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Inhibitory effect of chlorpromazine on the syndrome of hyperactivity produced by L-tryptophan or 5-methoxy-N,N-dimethyltryptamine in rats treated with a monoamine oxidase inhibitor

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Summary

1. The hyperactivity and hyperpyrexia produced by L-tryptophan in rats treated with a monoamine oxidase inhibitor was inhibited by chlorpromazine.

2. Chlorpromazine did not inhibit the increased rate of synthesis of brain 5-hydroxytryptamine (5-HT) produced by tryptophan loading.

3. Hyperactivity and hyperpyrexia were also produced by 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) in rats. Pretreatment with a monoamine oxidase inhibitor potentiated the hyperactivity response. Pretreatment of rats with *p*-chlorophenylalanine did not inhibit hyperactivity produced by 5-MeODMT.

4. Chlorpromazine inhibits hyperactivity caused by tryptophan or 5-MeODMT after monoamine oxidase inhibition either by competition with 5-HT or 5-MeODMT, respectively, at receptor sites or by physiological antagonism.

Introduction

When rats are treated with a monoamine oxidase (MAO) inhibitor and L-tryptophan they develop a stereotyped syndrome of hyperactivity accompanied by hyperpyrexia (Hess & Doepfner, 1961, Grahame-Smith, 1971). The production of this syndrome is dependent upon intact tryptophan hydroxylase activity, intact aromatic amino-acid decarboxylase activity and inhibition of monoamine oxidase. The development and severity of this syndrome are related not to the absolute concentration of brain 5-hydroxytryptamine (5-HT) but to the rate of accumulation of 5-HT in the brain. It has been suggested that when MAO is inhibited and an increased rate of brain 5-HT synthesis is produced by the administration of L-tryptophan, 5-HT 'spills over' and produces the excitatory changes in behaviour (Grahame-Smith, 1971). This same syndrome of hyperactivity is also produced in rats by certain hallucinogenic indolealkylamines, 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) being particularly potent (Ahlborg, Holmstedt & Lindgren, 1968; Grahame-Smith, 1971).

Chlorpromazine (cpz) inhibits the tremor produced in rats by 5-MeODMT (Ahlborg *et al.*, 1968) and its effect upon the syndrome of hyperactivity and increased rate of brain 5-HT synthesis produced by combined treatment with an MAO inhibitor and L-tryptophan has therefore been studied.

Methods

Male Sprague-Dawley rats (180–220 g) were used and all substances were given by intraperitoneal injection. Compounds were prepared for administration as previously described (Grahame-Smith, 1971). 5-MeODMT was dissolved in 0·9% saline. Rats were pretreated where indicated with chlorpromazine (Largactil: May & Baker Ltd.) 1 h before administration of tranylcypromine (20 mg/kg) and injections of L-tryptophan of 5-MeODMT were always given 30 min after the administration of tranylcypromine. The activity of groups of three rats was monitored on an Animex activity meter. Rectal temperatures were measured with a thermocouple probe.

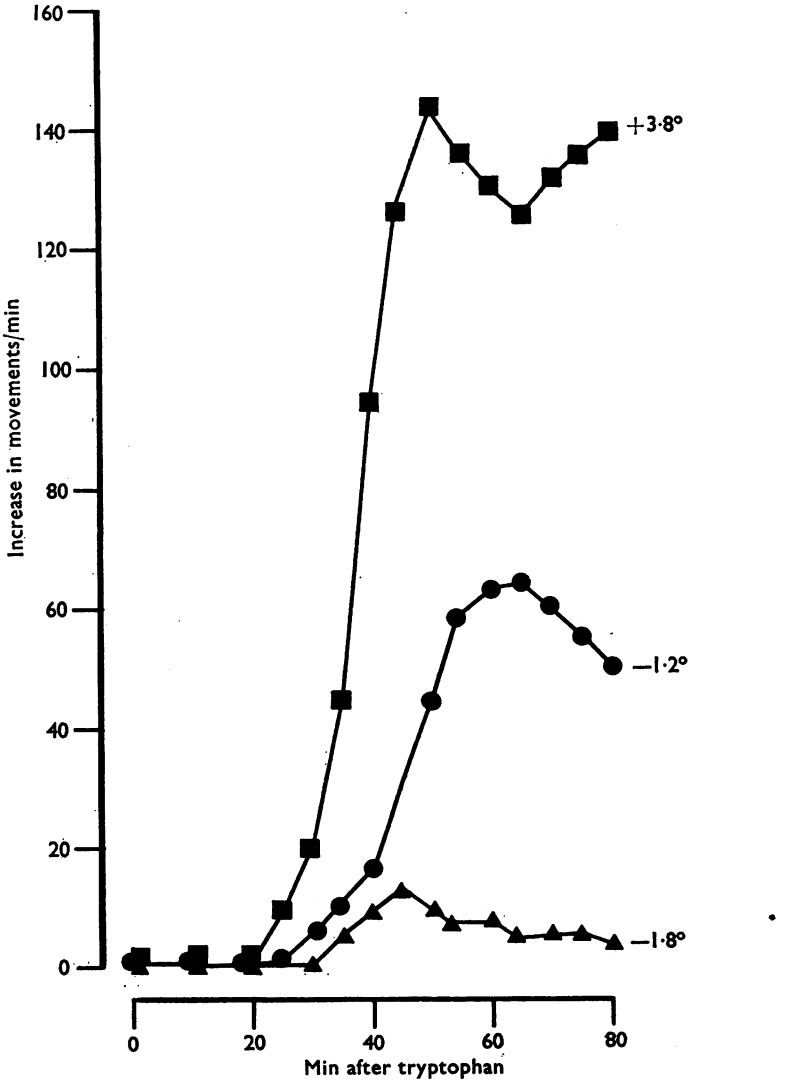

FIG. 1. Inhibitory effect of chlorpromazine (10 and 30 mg/kg) on the hyperactivity produced by tranylcypromine and L-tryptophan. Rats were given tranylcypromine (20 mg/kg) followed 30 min later by L-tryptophan (170 mg/kg). Chlorpromazine (CPZ) was given 1 h before administration of tranylcypromine. Three rats were monitored in each group. The change in average rectal temperature of the group, 80 min after tryptophan, is shown. ■——■, Controls; ●——●, CPZ (10 mg/kg); ▲——▲, CPZ (30 mg/kg).

Brain and plasma tryptophan concentrations were measured by the method described by Denckla & Dewey (1967) and brain 5-HT concentrations as described by Ansell & Beeson (1968).

Results

Inhibitory effect of chlorpromazine on the hyperactivity produced by MAO inhibition and L-tryptophan

When rats were given tranylcypromine (20 mg/kg) followed 30 min later by L-tryptophan (170 mg/kg) gross hyperactivity and hyperpyrexia were produced (Fig. 1). Chlorpromazine (10 mg/kg) given 1 h before tranylcypromine markedly decreased the hyperactivity and abolished the pyrexia (Fig. 1). A larger dose of chlorpromazine (30 mg/kg) increased the inhibition (Fig. 1).

Effect of chlorpromazine upon the increased concentration of brain 5-HT produced by MAO inhibition and L-tryptophan administration

Because the concentration of tryptophan in the brain is dependent upon the concentration of tryptophan in the plasma and because the concentration of brain tryptophan is a major factor determining the rate of synthesis of brain 5-HT (Grahame-Smith, 1971), the concentrations of brain and plasma tryptophan and brain 5-HT were determined 1 h after the administration of L-tryptophan (170 mg/kg) in rats pretreated either with tranylcypromine (20 mg/kg) alone, or chlorpromazine (10 mg/kg) and tranylcypromine.

Chlorpromazine had no significant effect upon either the increased concentrations of plasma and brain tryptophan or upon the increased concentrations of brain 5-HT found after treatment with tranylcypromine and L-tryptophan, under experimental conditions where hyperactivity and hyperpyrexia were inhibited (Table 1).

Effect of 5-MeODMT in producing hyperactivity and hyperpyrexia

Pretreatment with tranylcypromine greatly accentuated the hyperactivity produced by 5-MeODMT (Fig. 2), in addition to accentuating the fine body tremor (Ahlborg *et al.*, 1968). The degree of hyperactivity and hyperpyrexia was dependent upon the dose of 5-MeODMT up to a dose of approximately 5 mg/kg, above which increasing doses produced little increment in response (Fig. 3). Although the quality

TABLE 1. *Effect of chlorpromazine on the increased rate of accumulation of brain 5-HT produced by tranylcypromine and L-tryptophan*

Treatment	Plasma tryptophan (μg/ml±S.E.)	Brain tryptophan (μg/ml±S.E.)	Brain 5-HT (μg/g±S.E.)
Tranylcypromine and tryptophan	130·2±20	103·8±2·9	2·03±0·12
Chlorpromazine tranylcypromine and tryptophan	118·3±5·9	95·5±5·23	2·01±0·14

Groups of six rats were studied. One group received tranylcypromine (20 mg/kg) followed 30 min later by L-tryptophan (170 mg/kg). The other group received chlorpromazine (10 mg/kg) in addition, 1 h before the tranylcypromine. The measurements shown were made 1 h after administration of tryptophan.

of the syndrome produced by 5-MeODMT is identical to that produced by tranylcypromine and L-tryptophan, its onset is much faster usually occurring within 3 min of the administration of a submaximal dose of 5-MeODMT (for example 1 mg/kg). In the syndrome produced by tranylcypromine and L-tryptophan, the time of onset of hyperactivity cannot be shortened to less than 17 min even with very large doses of L-tryptophan. This presumably reflects the time taken for the concentration of

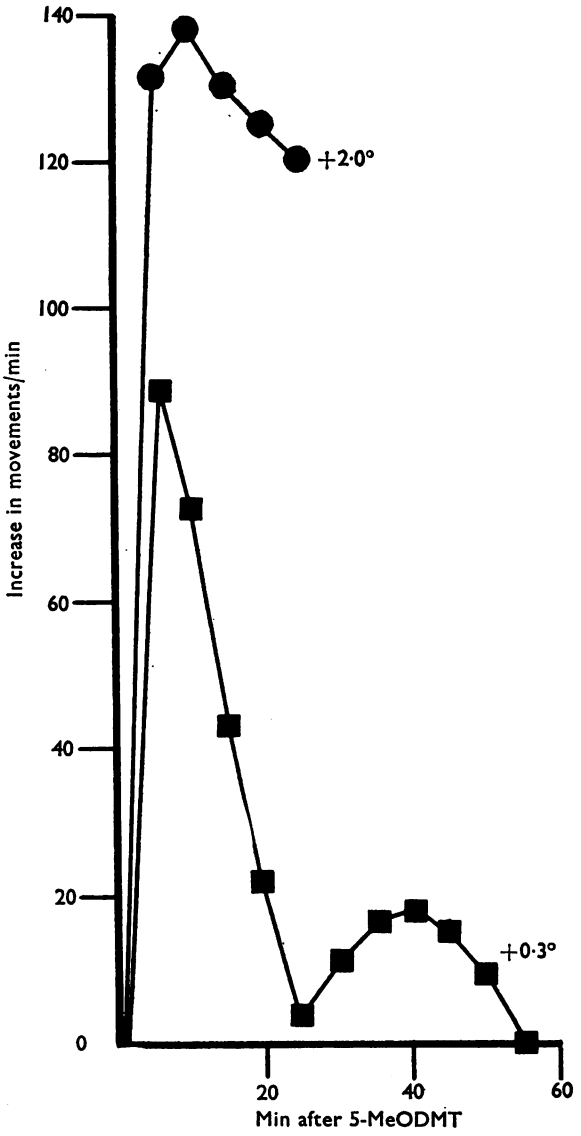

FIG. 2. Effect of monoamine oxidase inhibition in potentiating the hyperactivity and hyperpyrexia produced by 5-MeODMT. 5-MeODMT (1 mg/kg) was given at zero time. Tranylcypromine (TCP) had been given to one group of three rats 30 min before zero time. The animals given tranylcypromine and 5-MeODMT were grossly hyperactive; their rectal temperatures were taken after 25 min, those of the group not given tranylcypromine at 55 minutes. Change in mean rectal temperature is shown. ■——■, Controls; ●——●, rats given tranylcypromine.

brain tryptophan to build up, increase the rate of accumulation of brain 5-HT, and cause 'spill over' of brain 5-HT into functional activity (Grahame-Smith, 1971).

Effect of p-*chlorophenylalanine (PCP) upon the hyperactivity produced by 5-MeODMT in the presence and absence of MAO inhibition*

PCP inhibits the hyperactivity produced by L-tryptophan but not 5-hydroxytryptophan in rats pretreated with an MAO inhibitor (Grahame-Smith, 1971). This is due to the effect of PCP in inhibiting tryptophan 5-hydroxylation and thus the synthesis of 5-HT from L-tryptophan. PCP (300 mg/kg, daily for 2 days) did not inhibit the hyperactivity produced by 5-MeODMT either in the presence or absence of MAO inhibition with tranylcypromine. This experiment was done to explore the possibility that 5-MeODMT might produce its behavioural effects through the central release of 5-HT. Although 5-MeODMT is a probable substrate for MAO, thus explaining the potentiating action of MAO inhibition on its behavioural effects, it could be argued that 5-MeODMT released brain 5-HT, that this was responsible for the hyperactivity and that MAO inhibition potentiated the effects by stopping the inactivation of this released 5-HT. If this were the case then PCP, by inhibiting the synthesis of brain 5-HT and decreasing the concentration of 5-HT in brain, should attenuate the hyperactivity produced by 5-MeODMT. That it did not is indirect evidence that 5-MeODMT does not act by releasing brain 5-HT.

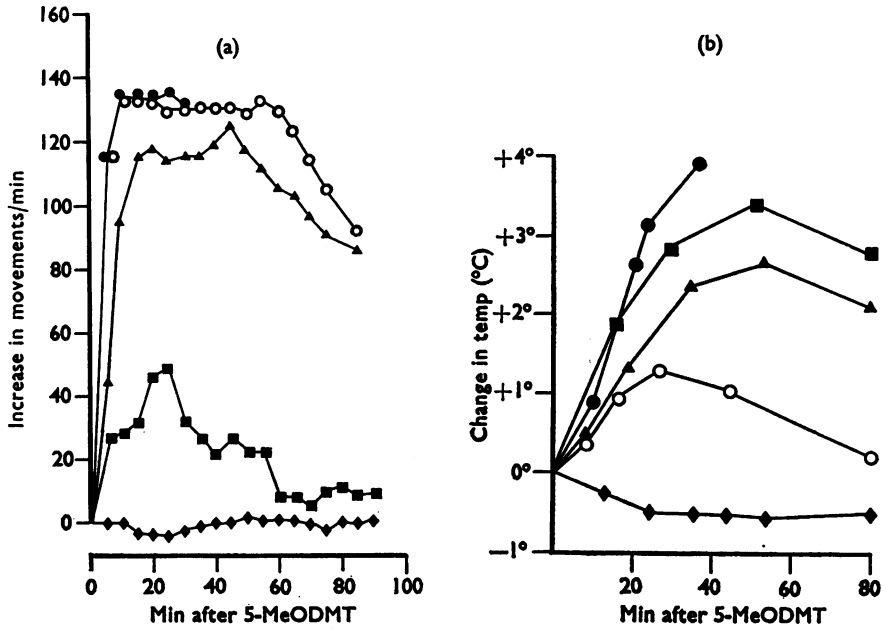

FIG. 3. Relationship between the dose of 5-MeODMT and the degree of hyperactivity and hyperpyrexia. Groups of three rats were pretreated with tranylcypromine (20 mg/kg) 30 min before various doses of 5-MeODMT. (a), Hyperactivity response. ●——●, 5-MeODMT (20 mg/kg); ○——○, 5-MeODMT (10 mg/kg); ▲——▲, 5-MeODMT (5 mg/kg); ■——■, 5-MeODMT (0·5 mg/kg); ◆——◆, control. (b), Temperature response to 5-MeODMT. ●——●, 5-MeODMT (20 mg/kg); ■——■, 5-MeODMT (10 mg/kg); ▲——▲, 5-MeODMT (5 mg/kg); ○——○, 5-MeODMT (0·5 mg/kg).

Inhibitory effect of chlorpromazine on the syndrome of hyperactivity produced by 5-MeODMT in rats pretreated with tranylcypromine

When chlorpromazine (10 mg/kg) was given 1 h before tranylcypromine (20 mg/kg), and 5-MeODMT (1 mg/kg) was given 30 min later, hyperactivity developed more slowly and lasted a shorter time than in rats not given chlorpromazine (Fig. 4). The increase in temperature was likewise inhibited in the rats treated with chlorpromazine (Fig. 4). Raising the dose of chlorpromazine to 30 mg/kg greatly increased the inhibition of hyperactivity and caused mild hypothermia (Fig. 4). The inhibitory pattern was similar to that seen with tranylcypromine and tryptophan (Fig. 1).

Discussion

The enzyme which decarboxylates 5-hydroxytryptophan is an aromatic L-amino acid decarboxylase with broad specificity (Lovenberg, Weissbach & Udenfriend, 1962) and for this reason it is possible that the administration of 5-hydroxytryptophan may lead to the formation of 5-HT in neurones normally synthesizing noradrenaline and dopamine, which contain the aromatic L-amino oxidase decarboxylase. It has been suggested (Grahame-Smith, 1967) that whether or not a

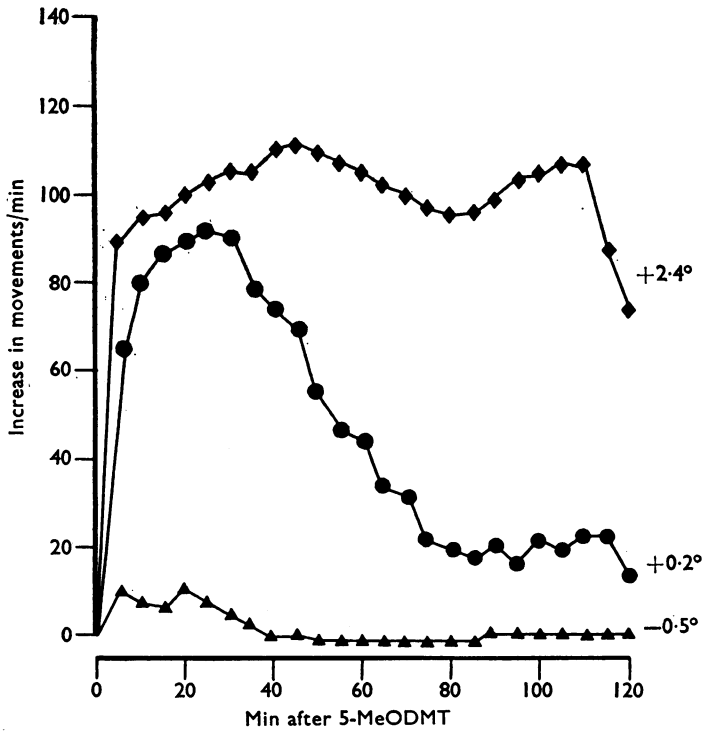

FIG. 4. Inhibitory effect of chlorpromazine on hyperactivity produced by 5-MeODMT in rats pretreated with tranylcypromine. Groups of three rats. CPZ was given 1 h before administration of tranylcypromine (20 mg/kg). Thirty minutes later (zero time in figure) 5-MeODMT (1 mg/kg) was administered. Activity of the animals was monitored for 120 min; change in mean rectal temperature of the rats is shown. ◆——◆, Controls; ●——●, CPZ (10 mg/kg); ▲——▲, CPZ (30 mg/kg).

catecholamine or 5-HT is made in a neurone, depends upon whether that neurone contains either tyrosine hydroxylase or tryptophan hydroxylase, these enzymes being specific for their respective substrates. It is probable therefore, though not yet proven, that monoamine oxidase inhibition and L-tryptophan loading increase the rate of brain 5-HT synthesis selectively in those neurones normally containing and synthesizing 5-HT and that the 'spill over' of 5-HT to produce hyperactivity occurs at sites at which 5-HT normally acts.

There are some apparent discrepancies in this hypothesis, because combined treatments in rats with monoamine oxidase inhibitors and 5-hydroxytryptophan produce an identical syndrome of hyperactivity, and MAO inhibition combined with L-dopa administration, also produces a similar but not identical excitatory behaviour pattern (Grahame-Smith: unpublished observation). The exact anatomical disposition of the increased synthesis and activity of 5-HT and dopamine may not be crucially important. Until the microscopic disposition of the monoamines is known, the discrepancies will not be understood.

The similarity of the syndrome of hyperactivity produced by tryptophan loading or 5-MeODMT in rats treated with a monoamine oxidase inhibitor, suggests that 5-HT and 5-MeODMT are acting at the same sites. PCP does not inhibit the behavioural effects of 5-MeODMT and this is indirect (but not absolute) evidence against 5-MeODMT acting through the release of endogenous brain 5-HT. 5-MeODMT has therefore been used in comparison with monoamine oxidase inhibition and tryptophan administration, to study the mechanism by which chlorpromazine acts to inhibit the syndrome of hyperactivity.

Chlorpromazine has a multitude of effects on various metabolic pathways and membrane permeabilities and in various systems it can act as a pharmacological antagonist of monoamines (see Gordon 1967). The problem as to whether the action of chlorpromazine in the brain is primarily due to an effect upon the synthesis or metabolism of 5-HT has been studied previously. The consensus of opinion (Ehringer, Hornykiewicz & Leshner, 1960; Morpurgo, 1962; Guldberg & Yates, 1968), is that any such effects on 5-HT synthesis or metabolism are most likely due to the hypothermia which chlorpromazine causes. The studies described here show quite definitely that in rats treated with MAO inhibitor and loaded with tryptophan, chlorpromazine does not significantly affect the increased rate of brain 5-HT synthesis resulting and that its inhibitory action on the hyperactivity caused by this treatment is in no way due to an alteration in the synthesis of brain 5-HT.

Attention must be directed therefore, to the effect of chlorpromazine either upon the release of 5-HT or upon its action. If the hypothesis is correct that 5-MeODMT acts directly at sites sensitive to 5-HT then the fact that the action of 5-MeODMT can also be inhibited by chlorpromazine is evidence against a mode of action of chlorpromazine by inhibiting the release of 5-HT. There is evidence that chlorpromazine can inhibit actions of 5-HT in various *in vitro* systems, but its action in the brain in this context is not fully understood. Bradley, Wolstencroft, Hösli & Avanzino (1966) suggested that chlorpromazine acts in the reticular activating system, reducing the function of this system in producing cortical arousal, and although the effect of chlorpromazine was thought by these workers to be mainly due to its inhibition of the excitation of neurones by noradrenaline, they did find that in two out of ten neurones, the excitant effect of 5-HT was inhibited by chlorpromazine. It is possible therefore, that the inhibitory effect of chlorpromazine on

the hyperactivity caused by tryptophan or 5-MeODMT after monoamine oxidase inhibition is due either to competition at receptor sites or to physiological antagonism. It has not been excluded that the inhibitory effects of chlorpromazine on hyperactivity produced by 5-HT and 5-MeODMT may be due to an inhibition of neuronal activity intermediate between the site of action of these amines and the areas in the brain which are finally responsible for the production of hyperactivity.

It is not yet clear what factors are primarily responsible for the hyperpyrexia produced by the treatments described. It may be due either to the gross hyperactivity and fine body tremor, which may have an effect comparable to that of shivering, or perhaps to some disturbance in hypothalamic temperature control produced by the action of high concentrations of 5-HT in the hypothalamus (by treatment with MAO inhibitors and L-tryptophan) or to a direct hypothalamic action of 5-MeODMT. The rise in temperature does not seem to be due to hyperactivity alone since chlorpromazine (10 mg/kg) whilst only partially inhibiting the hyperactivity due to tranylcypromine (20 mg/kg) and L-tryptophan (170 mg/kg) completely inhibited the rise in temperature (Fig. 1). On many occasions both with MAO inhibition and tryptophan or 5-MeODMT treatment this partial dissociation between hyperactivity and hyperpyrexia has been observed. Although it has not yet been measured, observation suggests that chlorpromazine inhibits the fine body tremor caused by these treatments more effectively than it inhibits the gross hyperactivity and perhaps the tremor is the most important factor causing the pyrexia. On the other hand, the findings suggest that chlorpromazine may inhibit the hyperpyrexia by an action separate from that by which the hyperactivity is reduced, and that the effect on rising temperature may represent a part of the direct mechanism by which chlorpromazine is known to produce hypothermia.

These studies with chlorpromazine have clinical relevance. It is likely that the great restlessness, excitation, motor activity and psychotic state produced by overdosage with MAO inhibitors (Reid & Kerr, 1969), and combinations of MAO inhibitors and tricyclic anti-depressants (Simmons, Carr & Ross, 1970) are due to a central action of monoamines, and chlorpromazine is effective treatment for such effects (Reid & Kerr, 1969). Whether or not the action of chlorpromazine to improve the schizophrenic state is due to an inhibition of a central monoamine action is a matter for conjecture.

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