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INFLUENCE OF PHENOTHIAZINE DERIVATIVES ON THE ACCUMULATION OF BRAIN AMINES INDUCED BY MONOAMINE OXIDASE INHIBITORS

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Abstract—The ability of chlorpromazine to antagonize the accumulation of serotonin in rat brain induced by tranylcypromine appeared to be dose-dependent. Only high doses of chlorpromazine reduced the increase due to administration of 1 mg tranylcypromine/kg intraperitoneally, but even 20 mg/kg i.p. failed to antagonize the maximal rise induced by 5 mg/kg i.p. of that inhibitor of monoamine oxidase (MAOI).

Perphenazine was as effective as chlorpromazine in inhibiting the MAOI-induced increase of serotonin and noradrenaline in brain. Trifluoperazine was less so, and significant inhibition was not observed with thioridazine. Pentobarbital also decreased the tranylcypromine-induced rise of brain serotonin. These results paralleled the degrees of hypothermia caused by the different compounds. When the chlorpromazine-induced fall in body temperature was prevented by keeping the rats at 36 °C the inhibition no longer occurred. Chlorpromazine failed to antagonize the increase of brain amines caused by tranylcypromine in rabbits, but body temperature appeared less affected in these animals than in rats.

These observations suggest that the hypothermia caused by chlorpromazine is an important factor in the reduction of the MAOI-induced accumulation of brain amines.

IN VIEW of the importance attributed to alterations in the content of brain serotonin and noradrenaline for the pharmacological activity of psychotherapeutic drugs, many experiments have been performed to discover whether chlorpromazine could affect the level of monoamines in the brain. Chlorpromazine did not produce significant changes in the levels of brain amines,[1, 2] despite some contradictory reports.[3, 4] It has been found instead to reduce the increase of serotonin and noradrenaline induced by iproniazid[1–3] as well as to interfere with the depletion caused by reserpine.[2, 4] It was suggested that chlorpromazine might affect permeability of the membrane of the monoamine storage granules.[5]

In order to determine whether these results were caused by a specific action of chlorpromazine or by a systemic effect resulting from the high dosages employed we studied: (1) the effects of varying doses of chlorpromazine and MAO inhibitors on the concentration of brain amines; (2) whether or not the ability to counteract the elevation of brain amines is a feature common to other phenothiazine derivatives used as

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tranquilizers; (3) the possibility that the drug-induced hypothermia is a factor in the moderating action of chlorpromazine on MAO inhibitors.

METHODS

Female albino Wistar rats weighing 170 to 220 g were injected intraperitoneally and killed by decapitation. Male albino rabbits, average weight 2·4 kg, were injected intravenously and sacrificed by air embolism. Unless otherwise specified, the animals were kept at a room temperature of about 24 °C. Individual rat brain minus the cerebellum and rabbit brain stem were analyzed for the serotonin and noradrenaline concentrations with spectrophotofluorimetric methods;[6, 7] Shore and Olin's procedure of extraction[7] was used with slight modifications for the simultaneous assay of both amines as suggested by Mead and Finger.[8] The results are expressed as a percentage of the average values of the controls, an equal number of untreated animals being analyzed in every experiment. Normal values, uncorrected for the recovery, were: serotonin 0·62 $\pm$ 0·01 ($n = 54$) and noradrenaline 0·30 $\pm$ 0·01 ($n = 49$) μg/g rat brain; serotonin 0·55 $\pm$ 0·02 ($n = 6$) and noradrenaline 0·37 $\pm$ 0·03 ($n = 6$) μg/g rabbit brain stem.

The following drugs were studied: iproniazid phosphate, tranylcypromine hydrochloride, chlorpromazine hydrochloride, perphenazine, trifluoperazine dihydrochloride, thioridazine hydrochloride, and pentobarbital sodium.

RESULTS

Table 1 summarizes the results obtained with varying doses of tranylcypromine and chlorpromazine on the serotonin accumulation in rat brain. The data obtained for noradrenaline were not included since they were not significant upon statistical analysis because of the variable rates of increase that we found after a single injection of that MAO inhibitor. The administration of 1 mg tranylcypromine/kg i.p. induced a rise of brain serotonin in 4 hr of the order of that found with 100 mg iproniazid/kg in 16 hr. An inhibition of the tranylcypromine-induced accumulation of serotonin similar to that reported for iproniazid was obtained by the pretreatment with 20 mg chlorpromazine/kg. This amount of chlorpromazine, however, was not effective against the larger dose of 5 mg tranylcypromine/kg. Significant inhibition was not found when the dosage of chlorpromazine was lowered to 5 mg/kg.

Table 2 reports comparative results of different phenothiazine derivatives administered before the MAO inhibitors iproniazid and tranylcypromine. Equally significant inhibitions in the increase of brain amines were obtained with chlorpromazine and perphenazine. A lesser degree of inhibition was observed with trifluoperazine whereas thioridazine proved to be almost ineffective at the same dose of 20 mg/kg i.p. Although all the animals injected with the phenothiazine derivatives appeared to be deeply sedated, a striking difference among the groups was noted in regard to body temperature. This was markedly decreased by chlorpromazine and perphenazine, but fell less with trifluoperazine and little or not at all with thioridazine. In this experiment the comparison of the changes in body temperature induced by the different compounds was only a casual observation, but it appeared to be in good agreement with data reported in the literature.[9]

In order to ascertain whether the fall in body temperature was responsible for the reduced increase of brain serotonin, the rats were maintained at 36 °C, which was described as the critical ambient temperature for the prevention of the chlorpromazine-induced hypothermia.[10] The changes in the concentration of brain serotonin are reported in Table 3, together with the average rectal temperatures for the single

TABLE 1. INTERACTION OF VARYING DOSES OF CHLORPROMAZINE AND TRANYLCYPROMINE ON THE INCREASE OF SEROTONIN IN RAT BRAIN

Treatment	No. of rats	Serotonin % of controls	*P*
Tranylcypromine 1 mg/kg i.p.	14	165 ± 6	
Tranylcypromine 1 mg/kg i.p. + chlorpromazine 20 mg/kg i.p.	14	136 ± 4	<0·01
Tranylcypromine 5 mg/kg i.p.	13	236 ± 7	
Tranylcypromine 5 mg/kg i.p. + chlorpromazine 20 mg/kg i.p.	13	227 ± 7	>0·10
Tranylcypromine 1 mg/kg i.p.	3	176 ± 10	
Tranylcypromine 1 mg/kg i.p. + chlorpromazine 5 mg/kg i.p.	3	159 ± 7	>0·10
Tranylcypromine 1 mg/kg i.p. + chlorpromazine 10 mg/kg i.p.	3	142 ± 3	<0·05
Tranylcypromine 1 mg/kg i.p. + chlorpromazine 20 mg/kg i.p.	3	140 ± 1	<0·01

Rats sacrificed 4 hr after administration of tranylcypromine.
Chlorpromazine injected 1 hr before tranylcypromine.
P versus tranylcypromine alone.

groups of rats. When the fall in body temperature was prevented, the inhibition by chlorpromazine no longer occurred. Moreover, the accumulation of brain serotonin caused by tranylcypromine was also antagonized by pentobarbital, another compound capable of inducing hypothermia in rats. (The content of brain serotonin was not significantly changed in rats injected only with pentobarbital and sacrificed 5 hr later.)

In rabbits 10 mg chlorpromazine/kg i.v. did not affect the increase of brain amines caused by a MAO inhibitor (Table 4), but measurements of rectal temperature showed no significant fall with this dose of chlorpromazine.

DISCUSSION

The results reported in this paper suggest that the underlying mechanism in the ability of chlorpromazine to antagonize the accumulation of monoamines in rat brain that is usually caused by MAO inhibitors is a systemic effect, probably the drug-induced hypothermia. In fact, only very large doses of chlorpromazine were found to be capable of counteracting the increase of serotonin in rat brain produced by the fast-acting MAO inhibitor, tranylcypromine. Such high doses caused a considerable

TABLE 2. INFLUENCE OF VARIOUS PHENOTHIAZINE DERIVATIVES ON THE INCREASE OF BRAIN AMINES INDUCED BY MAO-INHIBITORS

Treatment	No. of rats	Serotonin % of controls	*P*	Noradrenaline % of controls	*P*
Iproniazid 100 mg/kg i.p.	6	192 ± 8		161 ± 5	
Iproniazid 100 mg/kg i.p. + chlorpromazine 20 mg/kg i.p.	6	159 ± 6	<0·01	126 ± 3	<0·01
Iproniazid 100 mg/kg i.p. + perphenazine 20 mg/kg i.p.	6	160 ± 4	<0·01	141 ± 3	<0·01
Iproniazid 100 mg/kg i.p. + trifluoperazine 20 mg/kg i.p.	6	167 ± 6	<0·05	149 ± 5	>0·10
Iproniazid 100 mg/kg i.p. + thioridazine 20 mg/kg i.p.	6	174 ± 2	>0·05	144 ± 6	>0·05
Tranylcypromine 1 mg/kg i.p.	6	179 ± 4			
Tranylcypromine 1 mg/kg i.p. + chlorpromazine 20 mg/kg i.p.	6	141 ± 5	<0·01		
Tranylcypromine 1 mg/kg i.p. + perphenazine 20 mg/kg i.p.	6	140 ± 6	<0·01		
Tranylcypromine 1 mg/kg i.p. + trifluoperazine 20 mg/kg i.p.	6	146 ± 7	<0·01		
Tranylcypromine 1 mg/kg i.p. + thioridazine 20 mg/kg i.p.	6	169 ± 4	>0·10		

Rats sacrificed 16 hr after iproniazid and 4 hr after tranylcypromine.
Phenothiazine derivatives injected 1 hr before MAO inhibitors.
P versus MAO inhibitor alone.

TABLE 3. INFLUENCE OF PENTOBARBITAL AND CHLORPROMAZINE ON THE INCREASE OF BRAIN SEROTONIN INDUCED BY TRANYLCYPROMINE IN RATS

Treatment	No. of rats	Serotonin % of controls	*P*	Rectal temp.* °C
Tranylcypromine 1 mg/kg i.p.	12	171 ± 4		36·9
Tranylcypromine 1 mg/kg i.p. + pentobarbital 32 mg/kg i.p.	6	135 ± 4	<0·01	33·7
Tranylcypromine 1 mg/kg i.p. + chlorpromazine 20 mg/kg i.p.	12	143 ± 4	<0·01	33·7
Tranylcypromine 1 mg/kg i.p. + chlorpromazine 20 mg/kg i.p. (at 36 °C)	6	168 ± 4	>0·50	37·1

Rats sacrificed 4 hr after administration of tranylcypromine.
Pentobarbital and chlorpromazine injected 1 hr before tranylcypromine.
P versus tranylcypromine alone.
* Rectal temperature of control rats = 36·4 °C.

decline of body temperature, a decline that is likely to slow enzymatic reactions and thus reduce the synthesis of brain amines. When the fall in body temperature was prevented by keeping the rats in a room maintained at a temperature of 36 °C, the inhibition no longer occurred. In our experiments chlorpromazine and pentobarbital, in doses that counteracted the MAOI-induced accumulation of brain amines, showed no direct effect on the concentration of serotonin It can be presumed that there is normally a balance between formation and inactivation factors; therefore enzymatic changes related to the drug-induced hypothermia cannot manifest themselves.

TABLE 4. INFLUENCE OF CHLORPROMAZINE AND TRANYLCYPROMINE ON THE CONCENTRATION OF MONOAMINES IN RABBIT BRAIN STEM

Treatment	No. of rabbits	Serotonin %of controls	Noradrenaline % of controls	Rectal temp.* °C
Tranylcypromine 1 mg/kg i.v.	6	146 ± 4	122 ± 7	39·4
Tranylcypromine 1 mg/kg i.v. + chlorpromazine 10mg/kg i.v.	6	144 ± 6	117 ± 5	39·2

Rabbits sacrificed 4 hr after administration of tranylcypromine.
Chlorpromazine injected 1 hr before tranylcypromine.
* Rectal temperature of control rabbits = 38·9 °C.

The lack of inhibition by chlorpromazine of the tranylcypromine-induced rise of brain amines in rabbits is in agreement with the results of Pletscher and Gey,[2] who showed that no significant inhibition of the iproniazid-induced accumulation of brain serotonin and noradrenaline occurred in rabbits and guinea pigs. But whereas rats responded to the injection of 20 mg chlorpromazine/kg i.p. with a marked hypothermia, no significant fall was found in rabbits injected with 10 mg/kg i.v.; guinea pigs also have been reported to be particularly resistant to the hypothermic action of chlorpromazine.[11]

Correlations between the hypothermic effect and the ability to antagonize the MAOI-induced rise of brain amines were found for different phenothiazine derivatives. In fact thioridazine, less effective than chlorpromazine in lowering body temperature,[12] failed to counteract significantly the accumulation of serotonin and noradrenaline. Even trifluoperazine, in many aspects a more powerful compound than chlorpromazine, was less effective, in agreement with its weaker hypothermic action.[9]

Our results do not contradict the hypothesis advanced by Gey and Pletscher[5] that chlorpromazine acts by changing the permeability of the storage organelles for monomines, but it seems possible that such changes are mediated through the fall in body temperature.

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