Acta pharmacol. et toxicol. 1963, 20, 140–144.

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Effect of Chlorpromazine or Haloperidol on Formation of 3-Methoxytyramine and Normetanephrine in Mouse Brain

By

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(Received June 13, 1963)

Unlike reserpine and similar agents, chlorpromazine and haloperidol have little or no effect on the monoamine levels of the brain. Large doses of chlorpromazine have been shown to interfere with the effects of reserpine and also with the effects of MAO inhibitors on brain monoamine levels (EHRINGER, HORNYKIEWICZ & LECHNER 1960; PLETSCHER & GEY 1962). It appears that these effects are at least partly due to hypothermia (COSTA, GESSA & BRODIE 1962; PLETSCHER 1963).

The effect described in this paper appears at a lower dose level and is not secondary to hypothermia.

Methods

White mice of either sex, weighing about 20 g, were injected intraperitoneally with nialamide 100 mg per kg. After one hour various doses of chlorpromazine, haloperidol, phenoxybenzamine (bensylyt (NFN)) or promethazine were given intraperitoneally. The animals were then beheaded after another 3 hours. As controls, mice received nialamide alone. The animals were kept usually at room temperature (about 21°C), but in some experiments at 30°.

The brains of 9 mice were pooled and homogenized with 18 ml 0.4 N perchloric acid. After centrifugation 11 ml of the extract were taken for analysis of normetanephrine (NM) and 3-methoxytyramine (MT). The NM was determined by the method of CARLSSON & LINDQVIST (1962). As an extension of this method a second eluate was taken from the ion exchange column by means of 4 ml 0.1 N ammonia. This eluate contains the MT and is collected in a flask containing 0.15 ml 5 N HCl. The MT was oxidized by the method of CARLSSON & WALDECK (1963), except that our chromatographic technique did not necessitate addition of potassium chloride.

The remaining part of the extract (about 5 ml) was taken for assay of catecholami-

nes, noradrenaline (NA) by the method of BERTLER, CARLSSON & ROSENGREN (1958), and dopamine (DA) by the method of CARLSSON & WALDECK (1958), modified by CARLSSON & LINDQVIST (1962).

Results and Discussion

The accumulation in the brain of O-methylated catecholamine metabolites (MT and NM) brought about by nialamide was significantly enhanced by chlorpromazine and haloperidol (table 1). The threshold dose for this effect may be roughly estimated as 2.5 and 0.2 mg per kg for chlorpromazine and haloperidol, respectively. In the animals treated with tranquillizers, the accumulation of MT was possibly enhanced by elevation of the ambient temperature to 30°, but in our limited material the difference was not statistically significant. The levels of NA and DA were not significantly influenced by the tranquillizers. In the experiments with haloperidol the DA level was about 25% higher when the mice were kept at room temperature than at 30° ($p < 0.005$), but no corresponding effect was observed in the experiments with chlorpromazine. The material was not sufficient to permit any clear conclusions about the influence of temperature on the catecholamine levels.

A few experiments were performed with promethazine and phenoxybenzamine (= benzylyt (NFN)), but in them no significant effects were observed.

Our experiments show that low doses of chlorpromazine and haloperidol, *i.e.*, two major tranquillizers with different chemical structures but possibly similar modes of action, exerted a characteristic effect on the metabolism of brain catecholamines: they enhanced the accumulation of the O-methylated metabolites MT and NM brought about by MAO inhibition without influencing the levels of the catecholamines themselves. Among several possible mechanisms the most likely appears to be that chlorpromazine and haloperidol block monoaminergic receptors in brain: as is well known, they block the effects of accumulated 5-hydroxytryptamine and catecholamines when the accumulation is caused by MAO inhibitors or of the monoamine precursors 5-hydroxytryptophan and dihydroxyphenylalanine. It does not seem unreasonable to assume that this receptor blockade results in a compensatory activation of monoaminergic neurons. This activation should lead to increased release of monoamines and consequently to increased formation of monoamine metabolites. The reason why the increased monoamine release does not result in a decrease in monoamine levels may be that neuronal activation stimulates monoamine synthesis.

Experiments are in progress in this laboratory to elucidate the mecha-

Table 1.

Brain monoamine levels after various doses of chlorpromazine, haloperidol, phenoxybenzamine (bensylyt) or promethazine to mice pretreated with nialamide (100 mg per kg i.p.).

The drug was given one hour after the nialamide, and the mice were killed 3 hours later. In one experiment a second dose of promethazine was given 90 minutes before killing the animals. The values are means ± standard errors of the mean, expressed in µg/g brain. Figures in brackets indicate number of experiments. Each experiment was performed on 9 pooled brains.

Drug mg/kg i.p.	Noradrenaline	Dopamine	Normetanephrine	3-Methoxytyramine
		Chlorpromazine		
25	–[3]	–[3]	0.16 (1)	0.36 (1)
5	0.49 ±0.050 (2)	1.21 ±0.145 (2)	0.13 ±0.035 (2)	0.53 ±0.045 (2)
5[1]	0.62 ±0.045 (2)	1.16 ±0.119 (3)	0.13 ±0.032 (3)	0.67 ±0.083 (3)
5[2]	0.55 ±0.045 (4)	1.18 ±0.080 (5)	0.13[4] ±0.021 (5)	0.61[4] ±0.060 (5)
2.5	0.54 ±0.095 (2)	1.16 ±0.075 (2)	0.07 ±0.010 (2)	0.41 ±0.010 (2)
1	0.61 (1)	1.34 (1)	0.08 ±0.010 (2)	0.29 ±0.014 (2)
		Haloperidol		
15	0.54 ±0.090 (2)	1.21 ±0.155 (2)	0.07 ±0.065 (2)	0.51 ±0.000 (2)
1	0.57 ±0.023 (4)	1.14 ±0.019 (4)	0.09 ±0.005 (4)	0.61 ±0.096 (4)
1[1]	0.57 ±0.027 (3)	0.91 ±0.031 (3)	0.08 ±0.009 (3)	0.78 ±0.056 (3)
1[2]	0.57 ±0.016 (7)	– –	0.08[5] ±0.005 (7)	0.69[4] ±0.065 (7)
0.2	0.63 ±0.030 (2)	1.34 ±0.035 (2)	0.07 ±0.015 (2)	0.43 ±0.005 (2)
0.05	0.53 ±0.055 (2)	0.87 ±0.040 (2)	0.08 ±0.010 (2)	0.31 ±0.045 (2)
		Phenoxybenzamine		
20	0.65 ±0.033 (3)	1.05 ±0.200 (3)	0.08 ±0.044 (3)	0.30 ±0.014 (3)
		Promethazine		
20	0.89 (1)	1.31 (1)	0.04 (1)	0.25 (1)
20 + 10	0.64 (1)	1.39 (1)	0.08 (1)	0.25 (1)
		Control		
0	0.63 ±0.044 (5)	1.24 ±0.150 (5)	0.06 ±0.011 (7)	0.29 ±0.035 (7)
0[1]	0.62 ±0.037 (4)	1.00 ±0.092 (4)	0.04 ±0.015 (4)	0.34 ±0.036 (4)
0[2]	0.62 ±0.028 (9)	1.13 ±0.097 (9)	0.05 ±0.009 (11)	0.31 ±0.025 (11)

[1] The mice were kept at 30°.
[2] Total material (experiments at room temperature and 30° combined).
[3] Analysis not performed.
[4] Significantly ($p < 0.001$) higher than control.
[5] Almost significantly ($p < 0.025$) higher than control.

nism of the effect of chlorpromazine and haloperidol on the monoamines. It has been shown that chlorpromazine and haloperidol cause an increase in the levels of homovanillic and dihydroxyphenylacetic acids in rabbit brain (ANDÉN, ROOS & WERDINIUS 1963).

It is evident that the possible usefulness of metabolite levels as indicators of neuronal activity merits further investigation.

Summary

The accumulation in mouse brain of the catecholamine metabolites 3-methoxytyramine and normetanephrine brought about by inhibition of monoamine oxidase was found to be enhanced by small doses of chlorpromazine and haloperidol, but phenoxybenzamine (bensylyt) and promethazine were ineffective. Hypothermia could be eliminated as a causative factor.

The effect is, it is suggested, due to a compensatory activation of monoaminergic neurons after blockade of monoaminergic receptors.

Acknowledgements.

This research was supported by the United States Air Force under Grant No. AF-EOAR 63–14 and monitored by the European Office, Office of Aerospace Research; and by the Swedish Medical Research Council.

For generous supplies of drugs we thank Leo Ltd., Hälsingborg (Chlorpromazine and Haloperidol), Recip Ltd., Stockholm (Promethazine), Smith, Kline and French Labs., Philadelphia (Phenoxybenzamine), and the Swedish Pfizer Ltd., Näsbypark (Nialamide).

For technical assistance we thank Miss Anne-Charlotte Lilljeqvist and Miss Erica Weber.

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