Effects of Clozapine, Thioridazine, Perlapine and Haloperidol on the Metabolism of the Biogenic Amines in the Brain of the Rat

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Abstract. The effects of clozapine, thioridazine, perlapine and haloperidol on the metabolism of the biogenic amines in the brain of the rat have been investigated.

Haloperidol, perlapine and thioridazine induce catalepsy and enhance the turnover of DA in the striatum, as indicated by the dose-dependent increase in the DA-metabolites, HVA and DOPAC. These effects are due to blockade of dopaminergic transmission, haloperidol being far more potent than perlapine or thioridazine. Clozapine differs from these agents in that it elevates the concentration of striatal DA. The increase of the concentrations of HVA and DOPAC by clozapine is not accompanied by development of catalepsy. Therefore, clozapine seems to influence striatal DA by a mechanism other than DA-receptor blockade.

All four drugs enhance the turnover of NA in the brain stem. This effect is probably secondary to the blockade of NA-receptors. There was no correlation between the effects on NA-metabolism and the EEG-arousal inhibitory activities of these agents or their clinical antipsychotic effects.

Clozapine increase the concentration of 5-HT and 5-HIAA in the brain. This effect was not seen with the other drugs. Perlapine seems to enhance the turnover of 5-HT, whereas haloperidol reduces the 5-HT concentration. Thioridazine appears to have no effect on the metabolism of 5-HT.

Key words: Clozapine — Thioridazine — Perlapine — Haloperidol — Noradrenaline — Dopamine — Serotonin — Rat Brain.

Clozapine is a novel antischizophrenic drug that may be classified among the major tranquilizers with strong sedative activity. In man, this drug was found to lack extrapyramidal side-effects (Angst et al., 1971). The pharmacological profile suggests that clozapine is without effect on the nigro-striatal system, although electrophysiological evidence indicates that clozapine, in common with the cataleptogenic neuroleptics, increases the electrical excitability of neurons in the striatum, as shown by a decreased stimulation threshold and by a prolongation and increase of the amplitude of caudatum spindles (Stille, 1970; Stille et al., 1971; Sayers and Kleinlogel, 1973). Biochemical investigations in the rat revealed that clozapine, in contrast to the cataleptogenic neuroleptics, increases the content of dopamine (DA) in the striatum (Bürki, 1973; Bürki et al., 1973, 1974). An increase in the DA-turnover, as indicated by a raised homovanillic acid (HVA)-concentration in the striatum, was observed only after very high doses of clozapine (Bartholini et al., 1972). Therefore, the biochemical and pharmacological profiles of action of clozapine differ in important aspects from those of the cataleptogenic neuroleptics (Bürki, 1973; Bürki et al., 1974).

In subsequent neurochemical investigations, the results of which are reported here, it was thought of interest to compare clozapine not only with cataleptogenic neuroleptics but also with psychotropic drugs which are thought to act preferentially on the ascending reticular system (Stille et al., 1971). Therefore, we have included thioridazine, an established major tranquilizer, and perlapine, a sleep-promoting drug structurally related to clozapine but lacking antischizophrenic activity in man (Stille et al., 1973). Table 1 summarizes the main pharmacological actions of the agents used in the present study (Stille and Hippius, 1971; Stille et al., 1973). Clozapine, thioridazine and perlapine impair the arousal reaction induced by arecoline or by electrical stimulation of the reticular formation. Perlapine and thioridazine are weakly cataleptogenic, whereas clozapine has no cataleptogenic activity. Neither clozapine nor perlapine or thio-
ridazine provides protection against apomorphine-induced stereotypies. Haloperidol, on the other hand, is without effect on the arousal reaction, but is strongly cataleptogenic and protects against apomorphine stereotypies. It was, therefore, included in this study as an example of a typical cataleptogenic neuroleptic.

**Materials and Methods**

**Animals.** Male RAC rats weighing 120–170 g, obtained from Tierfarm AG, Sisseln, Switzerland, were used. The rats were kept in air-conditioned rooms at 25 °C and 50% air humidity and fed with Nafag pellets (Nafag AG, Gossau, Switzerland) and water ad libitum.

**Drugs.** Perlapine and clozapine were each dissolved in 1.25 molar equivalents of hydrochloric acid and diluted with water. Haloperidol solution (Cilag Chemie AG, Schaffhausen, Switzerland) was diluted with 0.9% sodium chloride solution. Thoridazine was dissolved in water. Treatment schedules are described in the respective tables.

**Biochemical Determinations.** After decapitation of the rats, the brains were dissected and the tissues were put on dry ice immediately. For the determination of DA, HVA, 3,4-dihydroxyphenylacetic acid (DOPAC), and noradrenaline (NA), the tissues were homogenized in 0.4 N perchloric acid, using a Polytron PT 20 OD S homogenizer (Kinematica, GmbH, Luzern), and the homogenates were centrifuged at 12,800 g for 10 min at 0–4 °C. The supernate was decanted and the pellet re-homogenized and re-centrifuged under the same conditions. The pooled supernates were used for analysis. From the perchloric acid supernates of the pooled striata of 5 rats, HVA was extracted with ether at pH 2, and re-extracted from the ether phase with tris buffer pH 8.5. Oxidation of HVA was effected with ferricyanide in ammonia solution (Andén, Roos, and Werdinuss, 1963). DOPAC was extracted from the perchloric acid supernates of the pooled striata of 2 rats with n-butyl acetate, and re-extracted from the n-butyl acetate phase with ethylenediamine solution for fluorimetric determination according to Spano and Neff (1971). DA was determined in the pooled striata of 4 rats after adsorption from the neutralized perchloric acid extract on aluminium oxide (Anton and Sayre, 1962), elution with diluted perchloric acid, and oxidation with periodate according to Anton and Sayre (1964). NA was determined fluorimetrically in the pooled brain stems of 4 rats after adsorption from the neutralized perchloric acid extract on aluminium oxide (Anton and Sayre, 1962), elution with diluted perchloric acid and oxidation with ferricyanide (Euler and Lishajko, 1961). The turnover rate of NA was assessed after blockade of the dopamine-β-hydroxylase with diethyldithiocarbamate (DDC), as described by Carlsson, Lindqvist, Fuxe, and Hökfelt (1966). DDC (500 mg/kg s.c.) was administered 15 min after the drugs, the rats were killed 2 hrs later and the NA content in the brain stem determined. For the determination of serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA), the pooled whole brains of 2 rats were homogenized in 0.1 N hydrochloric acid containing 0.5% ascorbic acid, the proteins precipitated by addition of zinc sulfate and sodium hydroxide, and the reaction mixtures were filtered to yield a clear solution which was used for the determinations. 5-HIAA was extracted from this solution at pH 1–2 with butyl alcohol and re-extracted from the butanol with diluted hydrochloric acid. 5-HT and 5-HIAA were determined fluorimetrically in the hydrochloric acid and phosphate buffer solutions, respectively, sufficient hydrochloric acid being added in each case to give 3N-solutions (Giacalone and Valzelli, 1969).

### Table 1

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Catalysis (rat)</th>
<th>Apomorphine Antagonism (rat)</th>
<th>Inhibition of Arousal Reaction (rabbit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clozapine</td>
<td>active</td>
<td>inactive</td>
<td>1.5</td>
</tr>
<tr>
<td>Perlapine</td>
<td>inactive</td>
<td>inactive</td>
<td>3.2</td>
</tr>
<tr>
<td>Thoridazine</td>
<td>inactive</td>
<td>inactive</td>
<td>2.4</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>inactive</td>
<td>inactive</td>
<td>2.1</td>
</tr>
</tbody>
</table>

* From Stille and Hippius, 1971

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8.5. Oxidation of HVA was effected with ferricyanide in ammonia solution (Andén, Roos, and Werdinuss, 1963). DOPAC was extracted from the perchloric acid supernates of the pooled striata of 2 rats with n-butyl acetate, and re-extracted from the n-butyl acetate phase with ethylenediamine solution for fluorimetric determination according to Spano and Neff (1971). DA was determined in the pooled striata of 4 rats after adsorption from the neutralized perchloric acid extract on aluminium oxide (Anton and Sayre, 1962), elution with diluted perchloric acid, and oxidation with periodate according to Anton and Sayre (1964). NA was determined fluorimetrically in the pooled brain stems of 4 rats after adsorption from the neutralized perchloric acid extract on aluminium oxide (Anton and Sayre, 1962), elution with diluted perchloric acid and oxidation with ferricyanide (Euler and Lishajko, 1961). The turnover rate of NA was assessed after blockade of the dopamine-β-hydroxylase with diethyldithiocarbamate (DDC), as described by Carlsson, Lindqvist, Fuxe, and Hökfelt (1966). DDC (500 mg/kg s.c.) was administered 15 min after the drugs, the rats were killed 2 hrs later and the NA content in the brain stem determined. For the determination of serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA), the pooled whole brains of 2 rats were homogenized in 0.1 N hydrochloric acid containing 0.5% ascorbic acid, the proteins precipitated by addition of zinc sulfate and sodium hydroxide, and the reaction mixtures were filtered to yield a clear solution which was used for the determinations. 5-HIAA was extracted from this solution at pH 1–2 with butyl alcohol and re-extracted from the butanol with diluted hydrochloric acid. 5-HT and 5-HIAA were determined fluorimetrically in the hydrochloric acid and phosphate buffer solutions, respectively, sufficient hydrochloric acid being added in each case to give 3N-solutions (Giacalone and Valzelli, 1969). The