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Binding Affinity of Levomepromazine and Two of its Major Metabolites to Central Dopamine and α-Adrenergic Receptors in the Rat

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Abstract. N-Monodesmethyl levomepromazine and levomepromazine sulfoxide have previously been found in higher plasma concentrations than the parent drug in patients who received oral doses of levomepromazine. In the present study levomepromazine, N-monodesmethyl levomepromazine and levomepromazine sulfoxide have been assayed for their binding affinity to rat striatal dopamine receptors and to α-adrenergic receptors in rat cortex, and compared with the potency of chlorpromazine and some of its metabolites in the same systems. Levomepromazine sulfoxide was relatively inactive in the dopamine receptor binding test but much more active in the α-adrenergic receptor binding test, where it had a binding affinity similar to 7-hydroxy chlorpromazine. Levomepromazine and N-monodesmethyl levomepromazine were active in both systems, having a slightly higher potency than chlorpromazine in the α-adrenergic binding test, and a somewhat lower potency than chlorpromazine in the dopamine receptor binding test. The results indicate that N-monodesmethyl levomepromazine may significantly contribute to the antipsychotic effects of levomepromazine while the sulfoxide metabolite lacks neuroleptic potency, and that both metabolites may contribute to the autonomic side-effects of the drug.

Key words: Levomepromazine metabolites – Dopamine receptor affinity – α-Adrenergic receptor affinity – Rat brain

The neuroleptics have long been regarded as a group of drugs where no correlations of practical utility between plasma levels and effects have been demonstrated (Lader 1976; Dahl 1979). As recently reviewed by Morselli (1981), a series of reports published during the last 2 years indicates that this situation is now about to change. A complicating aspect of the clinical pharmacology of the phenothiazine drugs is the uncertain significance of their different metabolites. It has been demonstrated that 7-hydroxy chlorpromazine (Kleinmann et al. 1980), thioridazine side-chain sulfoxide and thioridazine side-chain sulfone (Axelsson and Mårtensson 1977) have antipsychotic activity, by administration of the metabolites to psychiatric patients. It has also been shown that these metabolites may reach CSF levels in the same range as the CSF levels of the parent drug in patients who are treated with chlorpromazine (Sedvall 1981) or thioridazine (Mårtensson et al. 1980).

Levomepromazine (methotrimeprazine) has been used in Europe and Canada as a neuroleptic drug with pronounced sedative properties. The chemical structures of levomepromazine and two of its main metabolites are shown in Fig. 1. Pharmacokinetic studies with this drug have shown that patients generally have 1–2 times higher plasma concentrations of levomepromazine sulfoxide than of levomepromazine itself after repeated oral doses of the drug (Dahl 1976; Dahl et al. 1977). N-Monodesmethyl levomepromazine has also been identified in plasma from patients (Dahl and Garle 1977). The plasma concentrations of this metabolite have recently been measured in five psychiatric patients who were treated with levomepromazine tablets (Dahl, Bratlid and Lingjaerde, unpublished results). The plasma levels of monodesmethyl levomepromazine were about twice as high as the levels of the parent drug in four of the patients, and about 50% of the levomepromazine concentration in the fifth patient.

The clinical significance of the high plasma concentrations of the levomepromazine metabolites is unclear, since little is known about their pharmacological activity. Their cardiodepressive effects have been studied in isolated rat atria, where both metabolites appeared to be active (Dahl and Refsum 1976; Passwal et al. 1976). It was an unexpected finding that levomepromazine sulfoxide had a certain activity in this system, since chlorpromazine sulfoxide, which is

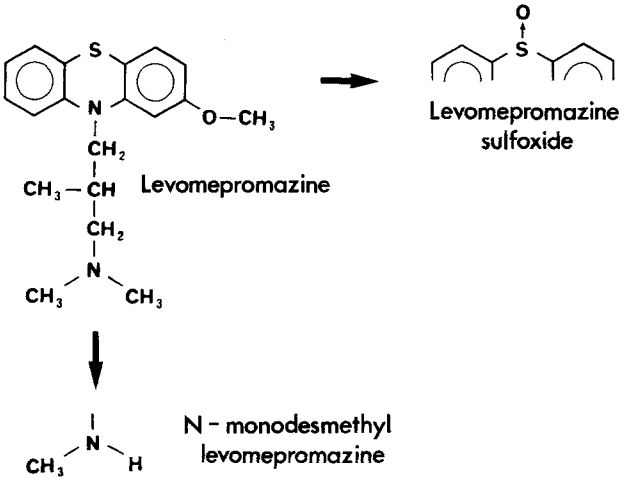

Fig. 1. Structures of levomepromazine and its two major non-polar metabolites in man

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known as a generally inactive metabolite, also had very low activity in the isolated rat atria.

The 3-dimensional molecular structures of levomepromazine sulfoxide and chlorpromazine sulfoxide have been examined by X-ray crystallography (Dahl et al. 1979). Levomepromazine sulfoxide had the same molecular conformation as the previously known structure of chlorpromazine, while chlorpromazine sulfoxide had another entirely different conformation of the side-chain. In view of this finding and of the high plasma levels of the two levomepromazine metabolites in patients, it was thought necessary to examine further their pharmacological activity.

It has been demonstrated that the neuroleptic potency of phenothiazine drugs correlates with their binding affinity to dopamine receptors in rat corpus striatal membranes, as assayed by their ability to compete with the binding of ^{3}H-haloperidol (Creese et al. 1976). This system has previously been used to assess the neuroleptic potencies of different chlorpromazine metabolites (Creese et al. 1978). The stereospecific binding sites for ^{3}H-haloperidol and ^{3}H-spiroperidol in rat corpus striatum appear to have identical pharmacological specificity (Leysen et al. 1978). In the present study the binding affinity of levomepromazine and its two major metabolites to rat striatal dopamine receptors has been assessed using ^{3}H-spiroperidol as the radioligand, and compared with the potencies of chlorpromazine and some of its metabolites in this system.

Excessive sedation and postural hypotension have been observed after oral doses of levomepromazine (Paradis 1959; Huot and Kirstof 1959; Lasagna and Dekornfeld 1961), and as recently reviewed by Creese (1978), these side-effects have been attributed to the blockade of central and peripheral α-adrenergic receptors. The binding affinity of drugs to central α-adrenergic receptors in the rat has been studied with (^{3}H) 2-([2′,6′-dimethoxy]phenoxyethylamino) methyl benzodioxan (^{3}H-WB 4101) as radioligand (Greenberg et al. 1976).

The present study also comprises an assessment of the binding affinity to α-adrenergic receptors in rat cortex of levomepromazine, chlorpromazine and some of their metabolites, measured with ^{3}H-WB 4101 as tracer.

Materials and Methods

Experimental Procedure. The experiments were performed essentially as previously described by Creese et al. (1978). Male Sprague-Dawley rats weighing 150–200 g were decapitated and the brain tissue dissected on ice. The tissues were homogenized by sonication at room temperature for 30–60 s in 20 ml 50 mM tris buffer, pH 7.7, and centrifuged at 48 000 *g* for 10 min. The pellet was washed twice by resuspension in 20 ml Tris buffer and centrifugation at 48 000 *g* for 10 min, and the final pellet was resuspended in 20 ml freshly prepared 50 mM Tris buffer, pH 7.6, containing 10 μM pargyline, 0.1% ascorbic acid, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$. The suspension was preincubated at 37° C for 10 min in order to inhibit amino-oxidases. Aliquots of 450 μl tissue suspension were added to test tubes which contained 25 μl solution of drug metabolite, and 25 μl tracer solution was added. The tubes were shaked on a whirlmixer and then incubated in a water bath. Additional experimental details are given in Table 1.

The incubations were interrupted by filtration on Whatman GF/B filters at 4° C, by a semiautomatic technique that has been previously described (Hall and Thor 1979). The filters were washed with Tris buffer at 4° C, and dissolved in Soluene by incubation at room temperature for 1 h. The radioactivity was measured by liquid scintillation counting in 10 ml Econofluor (New England Nuclear, Boston, Mass., USA). The total specific radioligand binding was determined by adding one of the specific displacers shown in Table 1 to the sample instead of drug or drug metabolite.

Table 1. Experimental details of α-adrenergic and dopaminergic receptor binding studies

Receptor type	α-Adrenergic	Dopaminergic
Brain region	Cortex	Striatum
Concentration of tissue suspension (mg wet weight/ml)	20	5
Tracer	^{3}H-WB 4101	^{3}H-spiroperidol
Tracer concentration (nM)	0.20	0.40
Specific activity (Ci/mmol)	24.4	25.14
Incubation time (min)	15	10
Incubation temperature (° C)	25	37
Specific displacer	Norepinephrine	+ Butaclamol
Displacer concentration (μM)	100	1.0

Tracers and Drug Metabolites. ^{3}H-spiroperidol and ^{3}H-WB 4101 were obtained from New England Nuclear, Boston, Mass., USA. Reference samples of the following phenothiazine derivatives were generously supplied by Rhône-Poulenc Industries, Paris, France: levomepromazine maleate, N-monodesmethyl levomepromazine maleate, levomepromazine sulfoxide (base), chlorpromazine sulfoxide (base), N-monodesmethyl chlorpromazine maleate, N-didesmethyl chlorpromazine maleate, N-monodesmethyl chlorpromazine sulfoxide (base) and 7-hydroxy chlorpromazine (base). The drugs and metabolites were dissolved in 0.1% ascorbic acid with addition of 1 N acetic acid when necessary, to a concentration of 2 mM, and then further diluted with 0.1% ascorbic acid such that 25 μl aliquots would give concentrations from 0.003–10 μM in a 0.5 ml sample.

Calculations. Each metabolite was added in different concentrations to four or five aliquots of tissue suspension, and the experiment was repeated on 2 or 3 different days so that each parameter was calculated from 8–14 experimental values. IC_{50}-values and regression coefficients were calculated by regression analysis from Hill plots, after a logit transformation.

Results

The concentrations of drugs and metabolites required to displace 50% of the specific radioligand binding are shown in Table 2. Chlorpromazine had higher binding affinity than all the other compounds tested in the dopamine receptor binding experiments. Levomepromazine had 40% lower potency than chlorpromazine in competing for ^{3}H-spiroperidol binding, and levomepromazine sulfoxide was virtually inactive, with about the same potency as chlorpromazine sulfoxide, in this system.

It is interesting to note, in view of the high blood levels of N-monodesmethyl levomepromazine in psychiatric patients, that this metabolite had 71% of the potency of levomepromazine in the dopamine receptor binding test (Table 2). The relative potencies of the different chlorpromazine metabolites in the ^{3}H-spiroperidol binding assay (Table 2) are in excellent agreement with the results of a similar study by Creese et al. (1978), who used ^{3}H-haloperidol as tracer.

Levomepromazine was twice as potent as chlorpromazine in competing for ^{3}H-WB 4101 binding in rat cortex (Table 2), and N-monodesmethyl levomepromazine was also active,

Table 2. Affinity of levomepromazine, chlorpromazine and metabolites for dopamine receptors and α-adrenergic receptors in rat brain. Values from two or three separate experiments were used in the calculations. IC_{50} = concentration of drug or metabolite required to displace 50% of specific radioligand binding. N = number of values. r = correlation coefficient

Compound	^{3}H-Spiroperidol striatum			^{3}H-WB 4101 cortex		
	IC_{50} (nM)	N	r	IC_{50} (nM)	N	r
Levomepromazine	57.3	8	−0.98	4.5	8	−0.94
N-Monodesmethyl levomepromazine	81.1	9	−0.94	5.8	11	−0.92
Levomepromazine sulfoxide	1110	10	−0.98	18.6	12	−0.82
Chlorpromazine	36.1	9	−0.97	8.8	13	−0.96
7-Hydroxy chlorpromazine	68.4	8	−0.96	23.5	9	−0.95
N-Monodesmethyl chlorpromazine	96.3	9	−0.98	15.1	14	−0.91
N-Didesmethyl chlorpromazine	354	9	−0.99	51.8	14	−0.92
Chlorpromazine sulfoxide	1480	10	−0.96	127	13	−0.85
N-Monodesmethyl chlorpromazine sulfoxide	1980	8	−0.99	261	13	−0.96

with about 80% of the potency of the parent drug, in this system. 7-Hydroxy chlorpromazine and chlorpromazine sulfoxide were, on the other hand, less active in the α-adrenergic binding test. Levomepromazine sulfoxide had 25% of the potency of levomepromazine, and slightly greater potency than 7-hydroxy chlorpromazine, in the ^{3}H-WB 4101 binding test.

Discussion

The low potency of levomepromazine sulfoxide in competing with ^{3}H-spiroperidol binding indicates that this metabolite lacks neuroleptic potency, and thus resembles the ring sulfoxides of other phenothiazine drugs in this respect.

It has previously been pointed out that ring sulfoxides of phenothiazine drugs which have a methoxy group or another electron-donating group as ring substituent in the 2-position have higher "bioreactivity" than the sulfoxides of chlorpromazine and other phenothiazines which have an electron-attracting group as ring substituent (Fenner 1974). This may explain why levomepromazine sulfoxide has higher potency than chlorpromazine sulfoxide in the ^{3}H-WB 4101 binding assay.

The potencies of neuroleptic drugs in competing for ^{3}H-WB 4101 binding sites correlate with their potencies in blocking norepinephrine toxicity (Creese 1978), but the clinical relevance of the ^{3}H-WB 4101 binding test is not clear. It should also be mentioned that the ^{3}H-WB 4101 binding assay measures the affinity for α_1-receptors only (Tanaka and Starke 1980), and the compounds which were examined in the present study may have different affinities for α_2-receptors. The data for levomepromazine sulfoxide which are shown in Table 2 do however indicate that this metabolite may contribute to the autonomic side-effects of levomepromazine.

Serum levels of neuroleptic drugs and their active metabolites have been measured by a radioreceptor assay based on their ability to reduce ^{3}H-spiroperidol binding to dopamine receptors in rat caudate membranes, and a significant correlation between neuroleptic serum levels and clinical response was found (Tune et al. 1980). The results from the present study show that the concentration of levomepromazine sulfoxide would not be measured by this technique, which may not be a disadvantage in studies of the relationship between serum levels and antipsychotic effects of levomepromazine. In view of the relatively high potency of levomepromazine sulfoxide in the ^{3}H-WB 4101 binding test, it seems that this metabolite should be assayed by other methods if the relationship between autonomic side-effects and plasma levels of levomepromazine is to be studied further.

The lower potency of levomepromazine compared to chlorpromazine in the ^{3}H-spiroperidol binding test is in accordance with the general view of levomepromazine as a relatively weak neuroleptic drug. The results shown in Table 2 indicate that N-monodesmethyl levomepromazine should also be regarded as pharmacologically active, but less potent than levomepromazine. The present and other studies with chlorpromazine and promazine have shown that phenothiazine drugs which have a dimethylamino group at the side chain generally retain much of their pharmacological activity after demethylation of one of the methyl groups.

In conclusion, the results of the present study indicate that both the sulfoxide and the N-monodesmethyl metabolite of levomepromazine may contribute to the autonomic side-effects of oral doses of the drug. N-Monodesmethyl levomepromazine may also significantly contribute to the antipsychotic effect of the drug, while levomepromazine sulfoxide seems to lack neuroleptic potency.

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