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In Vitro and *In Vivo* Receptor Binding and Effects on Monoamine Turnover in Rat Brain Regions of the Novel Antipsychotics Risperidone and Ocaperidone

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SUMMARY

Risperidone and ocaperidone are new benzisoxazol antipsychotics with particularly beneficial effects in schizophrenia. We report a comprehensive study on the *in vitro* and *in vivo* receptor binding profile of the new compounds, compared with haloperidol, and on the drug effects on monoamine and metabolite levels in various brain areas. The *in vitro* receptor binding and monoamine uptake inhibition profiles, comprising 29 receptors and four monoamine uptake systems, revealed that ocaperidone and risperidone bound primarily, and with the highest affinity thus far reported, to serotonin $5HT_2$ receptors (K_i values of 0.14 and 0.12 nM, respectively). Further, the drugs bound at nanomolar concentrations to the following receptors (K_i values, in nM, for ocaperidone and risperidone, respectively): α_1-adrenergic (0.46 and 0.81), dopamine D_2 (0.75 and 3.0), histamine H_1 (1.6 and 2.1), and α_2-adrenergic (5.4 and 7.3). In contrast, haloperidol showed nanomolar affinity for D_2 receptors (1.55) and haloperidol-sensitive σ sites (0.84) only. The *in vitro* binding affinity of ocaperidone, risperidone, and haloperidol for D_2 receptors was exactly the same when measured in membranes from rat striatum, nucleus accumbens, tuberculum olfactorium, and human kidney cells expressing the cloned human D_2 receptor (long form). *In vivo* binding in rats, using intravenous administration of [^{3}H]spiperone, revealed very potent occupation by ocaperidone and risperidone of $5HT_2$ receptors in the frontal cortex (ED_{50} of 0.04–0.03 mg/kg); in this respect, they were 6, 30, and 100 times more potent than ritanserin, haloperidol, and clozapine, respectively. Ocaperidone occupied D_2 receptors in the striatum and the nucleus accumbens with similar potency as did haloperidol (ED_{50} of 0.14–0.16 mg/kg). Risperidone revealed biphasic inhibition curves in the latter brain areas, indicating that [^{3}H] spiperone labeled both $5HT_2$ receptors (occupied by risperidone at <0.04 mg/kg) and D_2 receptors (risperidone ED_{50} of ~1 mg/kg). In the tuberculum olfactorium, $5HT_2$ and D_2 receptors were also distinguished with risperidone. The ED_{50} values for occupation of the latter were for ocaperidone and risperidone 2 times lower and for haloperidol 2 times higher than in the striatum. Ocaperidone, risperidone, and haloperidol readily increased the levels of the dopamine metabolites 3,4-dihydroxybenzene acetic acid and homovanillic acid in the striatum, the nucleus accumbens, the tuberculum olfactorium, and, to some extent, the frontal cortex. Dose-response curve shapes were markedly different; with ocaperidone maximal levels were reached at 0.16 mg/kg and maintained to 10 mg/kg); with risperidone the levels tended to increase continuously up to 10 mg/kg. Haloperidol produced dome-shaped curves (maximum at 0.16–0.63 mg/kg). The decline in dopamine metabolite levels at high haloperidol doses was accompanied by a 50% reduction in dopamine levels in the various brain areas. In contrast, ocaperidone and risperidone caused only 20% reduction of striatal dopamine levels and did not affect dopamine levels in the mesolimbic areas. The levels of the serotonin metabolite 5-hydroxyindole acetic acid remained exactly the same in all the brain areas with all three drugs. Hence, the feedback activation of monoamine turnover upon receptor blockade observed in the dopaminergic system appeared not to take place in the serotonergic system. Our findings *in vitro* and *in vivo* show that risperidone and ocaperidone both have an equal, extremely high, affinity for brain $5HT_2$ receptors. Potent $5HT_2$ antagonism is hypothesized to underly the therapeutic action of the drugs on negative symptoms in schizophrenia. Central D_2 receptor blockade is believed to have a major role in the effective treatment of positive symptoms of schizophrenia; in this respect ocaperidone is equipotent with haloperidol, whereas risperidone is somewhat less potent. Extrapyramidal side effects are inherent to central D_2 receptor blockade, but the side-effects can be attenuated by potent $5HT_2$ receptor blockade. The primary $5HT_2$ receptor affinity of both risperidone and ocaperidone probably underlies their reduced side-effect liability.

Risperidone was introduced as a novel antipsychotic with extremely potent serotonin $5HT_2$ and potent DA D_2 antagonistic properties. This was shown by *in vivo* pharmacological studies (1) and by *in vitro* receptor binding and receptor-

ABBREVIATIONS: 5HT, 5-hydroxytryptamine; NE, norepinephrine; DA, dopamine; DOPAC, 3,4-dihydroxybenzene acetic acid; HVA, homovanillic acid; 5HIAA, 5-hydroxyindole acetic acid; HPLC, high performance liquid chromatography.

mediated functional studies (2). The drug has revealed a clinical profile that is distinct from that of existing feuroleptics. Risperidone proved to be an effective antipsychotic, with low extrapyramidal side-effect liability and with beneficial effects on the negative symptoms of schizophrenia. The drug appeared to be particularly useful for outpatient maintenance therapy; in contrast to a usually observed aversion to neuroleptics, patients seemed to be satisfied with the risperidone medication and a high drug compliance was obtained (3–6). Ocaperidone, a close structural congener of risperidone (see Fig. 1), showed the same potent $5HT_2$ antagonistic properties as risperidone, but it was a more potent D_2 antagonist. The first clinical trials revealed that the drug was very effective for acute treatment of psychosis and agitation, with low incidence of extrapyramidal side-effects (7).

Today we can rely on more than 35 years of clinical experience with neuroleptics of several different chemical classes and with various pharmacological and biochemical profiles (8). Analysis of their profiles in relation to clinical effects has given substantial insight into the contribution of certain properties to therapeutic effects or side-effects; however, an ideal therapy has not been found and serious problems remain to be solved. Central D_2 receptor blockade, a common property of all neuroleptics, is generally accepted to underlie the successful treatment with these drugs of the positive symptoms of schizophrenia. Unfortunately, it also is the cause of the induction of extrapyramidal side-effects (8–10). Moreover, most neuroleptics are not effective for the treatment of the negative symptoms of the disease. A side-effect-free therapy for both positive and negative symptoms has remained the major goal for the treatment of schizophrenia. To this end, several different strategies have been proposed. Recently, much attention has been paid to the hypothesis that very potent $5HT_2$ antagonism, a conspicuous property of risperidone and ocaperidone, plays a role in the reduction of extrapyramidal side-effects and in the alleviation of the negative symptoms (2, 3). Several lines of evidence have supported this hypothesis. Potent serotonin $5HT_2$ antagonists in combination with neuroleptics have been found to reduce catalepsy in rats (11–13), to increase neuroleptic-induced dopamine turnover (14), and to reduce extrapyramidal symptoms in patients (15). Moreover, the selective $5HT_2$ antagonist ritanserin was shown to be a good therapeutic agent for the treatment of negative symptoms, generalized anxiety, and dysthymic disorders (16). Although several clinically available neuroleptics show combined D_2 and $5HT_2$ antagonism, the former effect predominates for almost all the drugs. Only pipamperone, one of the early butyrophenone derivatives, is primarily a $5HT_2$ antagonist (8, 17–19). This drug has been noted for its antiagitation properties, its unique resocializing effects, and its regulatory effect on disturbed sleep rhythms (20). Clozapine, which is frequently reported to cause no extrapyramidal symptoms or tardive dyskinesia, also shows higher affinity for $5HT_2$ than for D_2 receptors, but its primary effect is on histamine H_1 and α_1-adrenergic receptors and it is the only neuroleptic that is 5 times more potent as a muscarinic cholinergic antagonist than as a D_2 antagonist (8). Built-in antimuscarinic activity has also been proposed as a strategy to reduce extrapyramidal side-effects (21). Therefore, the specific clinical properties of clozapine cannot easily be ascribed to a particular pharmacological property. Another, still very much supported, theory in the search for a differentiation between antipsychotic effects and side-effects of drugs is that preferential blockade of mesolimbic D_2 receptors would favor the antipsychotic action over motor disturbances, which are believed to result from D_2 receptor blockade in the basal ganglia (22–24). To investigate this hypothesis, many studies in the past focused on the ability of neuroleptics to enhance DA utilization or turnover in mesolimbic versus striatal brain areas, an effect that is thought to be a direct consequence of D_2 receptor blockade (24–28).

This study of the brain regional receptor interactions of ocaperidone and risperidone, in comparison with the reference neuroleptic haloperidol, was undertaken in order to gain insight into the possible sites of action of the new drugs, which may underlie their beneficial clinical profile. We report the *in vitro* receptor binding and neurotransmitter uptake inhibition profile of ocaperidone and an updated profile of risperidone and haloperidol, comprising data on 29 different radioligand binding and four monoamine uptake tests. We investigated *in vitro* the binding affinities of the drugs for D_2 receptors in membrane preparations of rat striatum, nucleus accumbens, and tuberculum olfactorium and of the 293 human kidney cell line expressing a cloned human D_2 receptor gene (29). Using the *in vivo* receptor labeling technique with [^{3}H]spiperone (17, 30), we studied the occupancy of D_2 receptors in the striatum, the nucleus accumbens, and the tuberculum olfactorium and that of $5HT_2$ receptors in the frontal cortex by the aforementioned drugs and by ritanserin and clozapine, after their systemic administration to rats. Finally, we measured the levels of monoamines and their metabolites in striatal, mesolimbic, and various cortical brain areas after systemic treatment of rats with ocaperidone, risperidone, and haloperidol.

On the whole, our findings suggest that the primary $5HT_2$ receptor blockade concomitant with D_2 receptor blockade, in which risperidone shows a more pronounced difference than ocaperidone, is probably the important factor in the particular clinical profiles of the drugs.

Materials and Methods

***In vitro* radioligand receptor binding and neurotransmitter uptake inhibition profile.** Inhibition of radioligand receptor binding by ocaperidone, risperidone, and haloperidol was investigated in 29 different radioligand receptor binding assays. The receptor types, details on tissues, radioligands, and substances used to define nonspecific binding (blank), and assay conditions are summarized in Table 1. Inhibition by the drugs of the uptake of four neurotransmitters in rat brain synaptosome preparations was tested as described in Leysen *et al.* (2); details on the assay conditions are presented in Table 1. Assays were performed either with fresh or with frozen tissues, prepared as previously described (2). Drugs were added at appropriate concentrations, in such a way that the inhibition curves were defined by at least eight concentration points, measured in duplicate. All the experiments were repeated independently at least three times. Curves were analyzed either graphically or by computerized curve-fitting, using nonlinear regression analysis for one- or two-site curve-fitting (modifications of equations described in Ref. 31). The $-\log IC_{50}$ values (IC_{50} defined as the concentration producing 50% inhibition of specific radioligand binding or neurotransmitter uptake) were derived from individual curves. K_i values were calculated according to the method of Cheng and Prusoff (32), using the K_D values and the concentrations of the radioligands indicated in Table 1. The mean and standard deviation of the K_i values obtained in independent experiments were calculated. The *in vitro* dissociation rate of the ocaperidone-receptor complex was

TABLE 1

Assay conditions for radioligand binding and neurotransmitter uptake

	Tissue (species, area, membrane preparation, mg of tissue/assay)[a]	Assay conditions (buffer, volume, temperature, time)[b]	Labeled ligand (name, addition, concentration, K_D value)	Blank (compound, concentration)	References[c]
A. Receptor site					
α_1-Adrenergic	Rat, forebrain, TP, 25	A, pH 7.7, 2.2 ml, 25°, 20 min	[^{3}H]WB4101, 0.5 nM, 0.29 nM	Norepinephrine, 100 μM	Greenberg *et al.*, 1976
α_2-Adrenergic	Rat, cortex, TP, 10	A, pH 7.7, 1.1 ml, 25°, 30 min	[^{3}H]Clonidine, 3 nM, 1.8 nM	Norepinephrine, 2 μM	Greenberg *et al.*, 1976
β_2-Adrenergic	Human-β_1 cloned, *Escherichia coli*	L, pH 7.6, 1.1 ml, 37°, 60 min	[^{125}I]Iodocyanopindolol, 0.025 nM, 0.029 nM	Propranolol, 0.25 μM	Leysen *et al.*, 1990
β_2-Adrenergic	Human-β_2 cloned, *E. coli*	L, pH 7.6, 1.1 ml, 37°, 60 min	[^{125}I]Iodocyanopindolol, 0.025 nM, 0.017 nM	Propranolol, 0.25 μM	Leysen *et al.*, 1990
Cholinergic-muscarinic	Rat, striatum, TP, 5	D, pH 7.4, 1.1 ml, 37°, 20 min	[^{3}H]Dexetimide, 2 nM, 0.65 nM	Dexetimide, 2 μM	Laduron *et al.*, 1979
DA D_1	Rat, striatum, TP, 5	C, pH 7.7, 1.1 ml, 37°, 15 min	[^{3}H]SCH23390, 0.25 nM, 0.63 nM	Piflutixol, 1 μM	Schulz *et al.*, 1985
DA D_2	Rat, striatum, TP, 12.5	C, pH 7.6, 1.1 ml, 37°, 10 min	[^{3}H]Haloperidol, 2 nM, 1.3 nM	(+)-Butaclamol, 2 μM	Leysen *et al.*, 1978
Histamine H_1	Guinea pig, cerebellum, TP, 10	D, pH 7.4, 1.1 ml, 25°, 30 min	[^{3}H]Pyrilamine, 1 nM, 0.8 nM	Astemizole, 1 μM	Chang *et al.*, 1978, Laduron *et al.*, 1982
Histamine H_2	Guinea pig, striatum, TP, 25	C, pH 7.4, 1.1 ml, 25°, 30 min	[^{3}H]Tiotidine, 4 nM, 11 nM	Histamine, 1 mM	Gajtkowski *et al.*, 1983
Serotonin $5HT_{1A}$	Rat, hippocampus, TP, 10	B, pH 7.7, 1.1 ml, 37°, 10 min	[^{3}H]8-OHDPAT,[d] 0.5 nM, 0.84 nM	Spiroxatrine, 1 μM	Gozlan *et al.*, 1983
Serotonin $5HT_{1B}$	Rat, hippocampus, TP, 25	B, pH 7.7, 2.2 ml, 37°, 10 min	[^{3}H]5HT, (+spiroxatrine, 1 μM), 3 nM, 1.6 nM	LSD, 2 μM	Nelson and Taylor, 1986
Serotonin $5HT_{1C}$	Pig, choroid plexus, M+L+P, 5	G, pH 7.7, 0.55 ml, 37°, 30 min	[^{3}H]Mesulergine, 1 nM, 2.1 nM	Ritanserin, 1 μM	Pazos *et al.*, 1985
Serotonin $5HT_{1D}$	Calf, substantia nigra, TP, 20	J, pH 7.6, 2.2 ml, 37°, 30 min	[^{3}H]5HT (+8-OHDPAT, 30 nM, + mesulergine, 30 nM), 4 nM, 2.2 nM	Serotonin, 1 μM	Waeber *et al.*, 1988
Serotonin $5HT_2$	Rat, frontal cortex, M+L+P, 10	A, pH 7.6, 4.4 ml, 37°, 15 min	[^{3}H]Ketanserin, 1 nM, 0.42 nM	Methysergide, 1 μM	Leysen *et al.*, 1982
Serotonin $5HT_3$	N×G 108CC15 cells, 250,000 cells	K, pH 7.5, 0.55 ml, 37°, 60 min	[^{3}H]GR 65630, 2 nM, 1.7 nM	ICS 205-930, 1 μM	Hoyer and Neijt, 1987
Release site	Rat, striatum, TP, 10	A, pH 7.7, 1.1 ml, 37°, 15 min	[^{3}H]Ketanserin, (+BW501, 100 nM), 1 nM, 12.4 nM	Tetrabenazine, 1 μM	Leysen *et al.*, 1988a
Haloperidol-sensitive σ sites	Guinea pig, medulla oblongata, TP, 5	A, pH 7.7, 1.1 ml, 25°, 60 min	[^{3}H]Haloperidol, 1 nM, 0.58 nM	(+)-3PPP 10 μM	Largent *et al.*, 1984
μ-Opiate	Rat, forebrain, M+L+P, 20	A, pH 7.4, 2.2 ml, 37°, 15 min	[^{3}H]Sufentanil, 0.5 nM, 0.13 nM	Dextromoramide, 0.5 μM	Leysen *et al.*, 1983
TCP *N*-methyl-D-aspartate sites	Rat, hippocampus, TP, 10	H, pH 7.1, 1.1 ml, 25°, 30 min	[^{3}H]TCP, 2.5 nM, 10 nM	MK801, 1 μM	Vignon *et al.*, 1983, Honoré *et al.*, 1987
Ca^{2+} channel	Rat, cortex, M+L+P, 10	A, pH 7.7, 2.2 ml, 37°, 30 min	[^{3}H]Nitrendipine, 0.1 nM, 0.23 nM	Nifedipine, 0.1 μM	Murphy and Snyder, 1982
Na^+ channel	Rat, cortex, M+L, 10	I, pH 7.4, 1.1 ml, 37°, 30 min	[^{3}H]Batrachotoxin-B (+scorpion venom, 3 μg), 1 nM	R 19 116, 1 μM	Pauwels *et al.*, 1986
Benzodiazepine	Rat, forebrain, TP, 10	A, pH 7.4, 1.1 ml, 0°, 30 min	[^{3}H]Flunitrazepam, 0.3 nM, 2.4 nM	Clonazepam, 0.1 μM	Speth *et al.*, 1978
Cholecystekinin CCK-A	Rat, pancreas, TP, 1.25	M, pH 7.4, 0.55 ml, 25°, 20 min	[^{3}H]CCK8, 2 nM, 1.68 nM	CCK8, 1 μM	Chang and Lotti, 1986
Cholecystekinin CCK-B	Guinea pig, total cortex, TP, 6.25	M, pH 7.4, 0.55 ml, 37°, 30 min	[^{3}H]CCK8, 1 nM, 0.57 nM	CCK8, 1 μM	Chang and Lotti, 1986
Neurotensin	Guinea pig, forebrain, TP, 12.5	F, pH 7.4, 1.1 ml, 25°, 20 min	[^{3}H]Neurotensin, 1 nM, 7.1 nM	Neurotensin, 1 μM	Goedert *et al.*, 1984, Schotte *et al.*, 1986

TABLE 1—Continued

Assay conditions for radioligand binding and neurotransmitter uptake

	Tissue (species, area, membrane preparation, mg of tissue/assay)[a]	Assay conditions (buffer, volume, temperature, time)[b]	Labeled ligand (name, addition, concentration, K_D value)	Blank (compound, concentration)	References[c]
Substance P	Rat, forebrain, TP, 25	E, pH 7.4, 1.1 ml, 25°, 20 min	[^{3}H]Substance P, 1 nM, 0.3 nM	Substance P, 1 μM	Perrone *et al.*, 1983
Leukotriene D_4	Guinea pig, lung, M+L+P, 10	P, pH 7.4, 0.3 ml, 25°, 20 min	[^{3}H]Leukotriene D_4, 1 nM, 0.55 nM	ICI 198615, 10 μM	Mong *et al.*, 1985
Platelet-activating factor	Rabbit platelets, 500,000 platelets	O, pH 7.4, 0.3 ml, 25°, 30 min	[^{3}H]Platelet-activating factor, 0.5 nM, 0.55 nM	L-659989, 10 μM	Hwang and Lam, 1986
Thromboxane A_2	Human platelets, 500,000 platelets	N, pH 7.4, 0.3 ml, 37°, 30 min	[^{3}H]SQ 29548, 5 nM, 7.8 nM	ICI 159995, 10 μM	Halushka *et al.*, 1987
B. Uptake					
NE	Rat, hypothalamus, M+L, 10	Krebs, 1.1 ml, 25°, 5 min[e]	[^{3}H]NE, 3 nM	Desipramine, 1 μM	
DA	Rat, striatum, M+L, 2	Krebs, 1.1 ml, 25°, 2 min[e]	[^{3}H]DA, 3 nM	Cocaine, 10 nM	
Serotonin	Rat, cortex, M+L, 10	Krebs, 1.1 ml, 25°, 5 min[e]	[^{3}H]5HT, 3 nM	Clomipramine, 1 μM	
GABA	Rat, cortex, M+L, 2	Krebs, 1.1 ml, 25°, 2 min[e]	[^{3}H]GABA, (+1 μM GABA), 5 nM	(0°)	

[a] M+L+P, membranes from the heavy and light mitochondrial and microsomal fraction; TP, total particulate membrane fraction; M+L, heavy plus light mitochondrial fraction.

[b] Buffers: A, Tris·HCl, 50 mM; B, Tris·HCl, 50 mM; $CaCl_2$, 4 mM; C, Tris·HCl, 50 mM; NaCl, 120 mM; KCl, 5 mM; $MgCl_2$, 1 mM; $CaCl_2$, 2 mM (ascorbic acid, 0.1%; pargyline, 2 μM for DA D_2 only); D, Na-K phosphate, 50 mM. E, Tris·HCl, 50mM; bovine serum albumin, 0.1%; bacitracine, 0.2 mM; KCl, 5 mM; $MnCl_2$, 3 mM; F, Tris·HCl, 50 mM; bovine serum albumin, 0.1%; orthophenanthroline, 0.5 mM; EDTA, 1 mM; G, Tris·HCl, 50 mM; $CaCl_2$, 4 mM; ascorbic acid, 0.1%; H, Tris·HCl, 5 mM; L-glutamate, 100 μM; I, HEPES-Tris, 50 mM; choline chloride, 130 mM; KCl, 5.4 mM; $MgSO_4$, 0.8 mM; glucose, 5.5 mM; J, Tris·HCl, 50 mM; $CaCl_2$, 4 mM; ascorbic acid, 0.1%; pargyline, 2 μM; K, Tris·HCl, 20 mM; NaCl, 154 mM; L, Tris·HCl, 10 mM; NaCl, 100 mM; M, Tris·HCl, 10 mM; NaCl, 120 mM; $MgCl_2$, 10 mM; bovine serum albumin, 0.1%; EGTA, 1 mM; soybean trypsin inhibitor, 50 μg/ml; bacitracine, 0.14 mM; phenylmethanesulfonyl fluoride, 10 μM; N, Tris·HCl, 50 mM; NaCl, 120 mM; glucose, 5 mM; indomethacin, 10 μM; O, Tris·HCl, 10 mM; $MgCl_2$, 10 mM; bovine serum albumin, 0.25%; P, Tris·HCl, 10 mM; $MgCl_2$, 10 mM; $CaCl_2$, 10 mM; cysteine, 5 mM; glycine, 5 mM; bovine serum albumin, 0.25%; Krebs-(Henseleit) medium; $MgSO_4$, 1.17 mM; NaCl, 118.1 mM; KCl, 4.70 mM; KH_2PO_4, 1.17 mM; $NaHCO_3$, 25 mM; D-(+)-glucose, 10 mM; $CaCl_2$, 2.5 mM.

[c] The indicated references are not included in the reference list of this article; they can be obtained from the authors on request.

[d] 8-OHDPAT, 8-hydroxydipropylamindetralin; (SD, lysergic acid diethylamide; 3-PPP, propyl-3-(3-hydroxyphenyl) piperidine, CCK8, sulphated cholecystokinin octapeptide; GABA, γ-aminobutyric acid; HEPES, 4-(2-hydroxyethyel piperazine-1-ethanesulfonic acid; EGTA, 2-ethyleneglycol-bis-(2-aminoethyl) tetraacetic acid.

[e] Tissue plus inhibitor are preincubated for 5 min at 25° before the radioactive drug is added.

measured for the $5HT_2$, D_2, α_1, α_2, and H_1 receptor in a similar way, as previously described (2).

D_2 receptor binding in various rat brain tissues and in the 293 human embryonic kidney cell line expressing cloned human D_2 receptors. The striatum, nucleus accumbens, and tuberculum olfactorium of female Wistar rats (150 g) were dissected immediately after decapitation. Total particulate membrane preparations were prepared as previously described (2). The final membrane pellet was stored at −80°. For radioligand binding assays, the membranes were thawed and suspended in buffer C (see Table 1) at a dilution of 2000 (volumes per original wet weight of tissue) for striatal tissue (yielding 5 μg of protein/ml, final concentration), a dilution of 1500 for nucleus accumbens (6 μg of protein/ml), and a dilution of 1000 for tuberculum olfactorium (10 μg of protein/ml).

The 293 human kidney cells, transfected with the st3 clone of the human D_2 receptor gene, were cultured as described (29). After a maximum of 17 passages, the cells from each Falcon 175 flask were harvested in 1 ml of Dulbecco's modified Eagle medium with 10% dimethyl sulfoxide and were stored at −80°. For radioligand binding, the cells were thawed, suspended in 30 ml of 50 mM Tris·HCl, pH 7.7, and centrifuged for 10 min at 36,000 × g, in a refrigerated Sorvall centrifuge. The pellet was washed by rehomogenization and recentrifugation. The final pellet was suspended in 40 ml of buffer C (see Table 1), corresponding to 40 μg of protein/ml.

For radioligand binding to D_2 receptors in these dilute tissue preparations, 2′-[^{125}I]iodospiperone (33) was used in the additional presence of 100 nM BW501, to occlude $5HT_2$ receptors in the rat brain tissues. Nonspecific binding was defined in assays in the presence of 1 μM halopemide, a specific dopamine D_2 antagonist (8). Incubation mixtures were composed of 0.5 ml of tissue preparation, 0.025 ml of 2′-[^{125}I] iodospiperone and BW501 diluted in buffer, and 0.025 ml of drug or blank substance in 10% ethanol or solvent. For concentration binding curves, 2′-[^{125}I]iodospiperone was added at 12 concentrations between 0.01 and 0.4 nM. Data were analyzed in Scatchard plots, and K_D and B_{max} values were derived as described (34). For inhibition by drugs, 0.1 nM 2′-[^{125}I]iodospiperone was used and drugs were added at the concentration shown in the figures. Inhibition curves were analyzed by computerized curve-fitting, as described above.

***In vivo* radioligand receptor binding.** Male Wistar rats (200 g) were treated subcutaneously with various dosages (0.01–10 or 2.5–40 mg/kg; see Figures) of the drugs dissolved in saline (injection of 1 ml of drug solution/100 g of body weight) or with saline (control); 1 hr thereafter the rats received 1 μg/kg (5–10 μCi) [^{3}H]spiperone by intravenous injection in the tail vein. The rats were sacrificed by decapitation 1 hr after the [^{3}H]spiperone injection; the striatum, the nucleus accumbens, the tuberculum olfactorium, the frontal cortex, and the cerebellum were immediately dissected. The tissues were cooled on ice, weighed, and dissolved in 10 ml of Instagel II (Packard Canberra, Downers Grove, IL), in plastic counting vials. After 48 hr the radioactivity was counted in a Packard Tri Carb liquid scintillation counter; data were expressed in dpm, using external standard counting and referring to a quenched standard curve (Packard, Downers Grove, IL). The counted radioactivity was converted to pg of [^{3}H]spiperone/mg of tissue. Four to six animals were treated at each drug dosage. For each drug and brain area, the values were averaged and graphically plotted versus the logarithm of the drug dosages. On each graph, values measured in the cerebellum were plotted; labeling in the cerebellum was taken as an indication of nonspecific tissue labeling. From the graphs, we derived the drug dosage that inhibited 50% of the specific labeling by [^{3}H]spiperone in a particular brain area.

Determination of monoamine and metabolite levels in rat brain tissue. Male Wistar rats (200 g) were treated subcutaneously with various dosages of the drugs (0.01–10 mg/kg) and sacrificed by decapitation after 2 hr. The brains were immediately removed and cooled on ice; the striatum, nucleus accumbens, tuberculum olfactorium, frontal cortex, parietal cortex, and occipital cortex were dissected rapidly. Immediately after the dissection, the tissues were weighed and frozen in liquid nitrogen. The samples were lyophilized for 48 hr and stored at −80°.

The lyophilized tissue samples were weighed and homogenized with an Ultra-Turrax homogenizer for 2 × 10 sec, in 1 ml of ice-cold 0.4 N $HClO_4$; 0.25 ml of the homogenate was kept for protein determination. The remaining homogenate was centrifuged (Eppendorf centrifuge) for 1 min at 14,000 × g. Forty microliters of the supernatant were directly injected into a HPLC apparatus, and the amounts of monoamines (NE, DA, and 5HT) and of their metabolites (DOPAC, HVA, and 5HIAA) were measured in one run. The instrument used was a Varian HPLC model 5060, equipped with a microcomputer-controlled reciprocating single-piston pump system. The electrochemical detector was a dual BAS model LC 4B/17 from Bioanalytical Systems, equipped with a glassy carbon electrode. The detector potentials were set at +0.72 V and +0.6 V, at 5 namp, versus the reference electrode Ag/AgCl. Elution time was <30 min. All chromatograms were monitored on a multitasking microcomputer data system (Varian CD5654). The raw data were stored on floppy disks for subsequent calculation. Narrow-bore stainless steel columns (25 cm × 4 mm inner diameter) filled with spherical Li-Chrospher RP particles of 5 μm, with a pore size of 60 Å, were used throughout this study. The efficiency ranged from 70,000 to 100,000 theoretical plates/m. The optimum flow rate was 0.9 ml/min. The columns, stainless steel Li ChroCart cartridges filled with Superspher 60 RP-8, were obtained from Merck AG. The elution solvent was prepared from an aqueous mixture of 0.2 M sodium formate, 0.5 mM EDTA, 5 mM heptanesulfonic acid (brought to pH 3.75 with formic acid), and acetonitrile/methanol (60:40 v/v). To 1 liter of the aqueous buffer solution, 115 ml of the organic solvent mixture were added, and the final solution was degassed by ultrasonication. The system was calibrated several times each day with a known amount of different catecholamines and their metabolites.

Relative retention times, related to serotonin, and minimum detectable quantities were as follows: NE, 0.17, 1.8 pg; DOPAC, 0.27, 1.7 pg; DA, 0.38, 1.4 pg; 5HIAA, 0.50, 1.0 pg; HVA, 0.60, 3.5 pg; and 5HT, 1.0, 1.6 pg. The absolute retention time of 5HT was 25 min. For protein determination, the sample of the homogenate was diluted with 1 M NaOH and assayed according to the method of Lowry *et al.* (35).

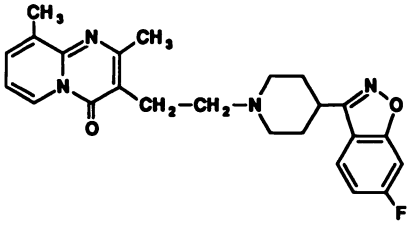
Materials. Labeled drugs listed in Table 1 were obtained from New England Nuclear, DuPont de Nemours (Dreieich, Germany), Amersham (Buckinghamshire, UK), or Janssen Biotech (Olen, Belgium); the specific radioactivity was between 14 and 110 Ci/mmol for tritiated compounds and maximally 2000 Ci/mmol for ^{125}I-labeled compounds. All radioactive ligands were checked for purity every 4 weeks, by thin layer chromatography; purity was required to be >98%. 2′-[^{125}I]Iodospiperone was synthesized and purified by Dr. J. Mertens (Cyclotron, Free University of Brussels, Jette, Belgium); the initial specific radioactivity was 1975 Ci/mmol.[1] Blank substances were generously provided by the companies of origin. Ocaperidone (R 79 598), risperidone (R 64 766), haloperidol (R 1625), and ritanserin (R 55 667) are products from Janssen Pharmaceutica (Beerse, Belgium); clozapine was donated by Sandoz (Basel, Switzerland). The 293 human kidney cells, transfected with the st3 clone of the human D_2 receptor gene encoding the 443-amino acid receptor protein, were kindly provided by Dr. Peter Seeburg (ZMBH, Heidelberg, Germany).

Results

The chemical structures of and physiocochemical data on ocaperidone (R 79 598), risperidone (R 64 766), and haloperidol (R 1625) are shown in Fig. 1. Ocaperidone and risperidone are both benzisoxazol derivatives and are structurally distinct from the butyrophenone haloperidol. The three compounds are li-

[1] Leysen, J. E., W. Sommerin, G. Mertens, P. J. Pauwels, M. Ewert, and P. Seeburg. Comparison of *in vitro* binding properties of a series of dopamine antagonists and agonists for cloned human D_{2S} and D_{2L} receptors and for D_2 receptors in rat striatol and mesolimbic tissues, using 2′-[^{125}I]iodospiperone. Submitted for publication.

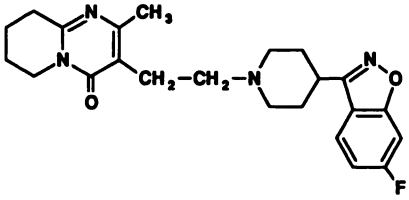
Ocaperidone
R 79598



log P = 4.13
pK_a = 8.3
degree of ionisation at pH 7.4 = 88.82 %

3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl] ethyl]-2,9-dimethyl-4*H*-pyrido[1,2-a]pyrimidin-4-one.

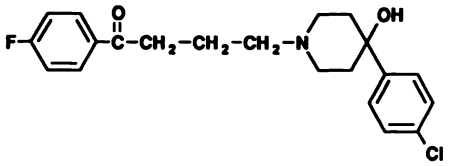
Risperidone
R 64766



log P = 3.04
pK_a = 8.24
degree of ionisation at pH 7.4 = 87.37 %

3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4*H*-pyrido[1,2-a]pyrimidin-4-one.

Haloperidol
R 1625



log P = 4.30
pK_a = 8.66
degree of ionisation at pH 7.4 = 94.79 %

4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butane.

Fig. 1. Chemical structures and physicochemical data for ocaperidone, risperidone, and haloperidol.

pophilic and basic. At the physiological pH of 7.4, they are 87–95% ionized; at this pH they are 150–1500 times more soluble in octanol than in water.

***In vitro* receptor binding profile.** Ocaperidone, risperidone, and haloperidol were tested *in vitro* for inhibition of radioligand binding to tissue homogenates and for inhibition of radiolabeled neurotransmitter uptake in rat brain synaptosome preparations in the various tests listed in Table 1. The apparent inhibition equilibrium constants (K_i values or IC_{50} values) are shown in Table 2. All the data for ocaperidone are original values. Some of the data for risperidone and haloperidol were reported before (2); however, the present table is extended by 13 radioligand binding models, compared with the previously published one.

The benzisoxazol derivatives ocaperidone and risperidone both bind primarily and with the same subnanomolar affinity to the $5HT_2$ receptor. Further, they show subnanomolar to low nanomolar affinity for α_1, D_2, and H_1 receptors. Haloperidol binds primarily and with low nanomolar affinity to the D_2 receptor and to the haloperidol-sensitive σ sites.

With regard to serotonin receptor subtype interactions, ocaperidone shows at least 50-fold and risperidone at least 400-fold higher affinity for the $5HT_2$ receptor than for any of the other subtypes. Ocaperidone differs from risperidone by its affinity in the high nanomolar range for $5HT_{1A}$ and $5HT_{1D}$ receptors. Haloperidol binds 16 times more weakly to $5HT_2$ than to D_2 receptors and shows virtually no interaction with $5HT_1$ receptor subtypes and none with $5HT_3$ receptors.

The affinity of ocaperidone and risperidone for the α_1 receptor is 3- and 7-fold weaker and for the α_2 receptor is 40- and 60-fold weaker, respectively, than their affinity for the $5HT_2$ receptor. The ratio for haloperidol between its primary activity (D_2 interaction) and α_1 receptor interaction is 7-fold; haloperidol does not interact with α_2 receptors. The three compounds have extremely weak or no interactions with β-adrenergic receptors.

With regard to D_2 receptor interaction, ocaperidone is 2 times more potent and risperidone 2 times less potent than haloperidol. The D_2 interaction of ocaperidone and risperidone is 5.3- and 25-fold weaker, respectively, than their binding affinity for $5HT_2$ receptors. The three compounds have only weak interactions with D_1 receptors.

Ocaperidone and risperidone have 11 and 18 times weaker affinity, respectively, for H_1 receptors than for $5HT_2$ receptors. Haloperidol binds only very weakly to H_1 receptors. The compounds bind only very weakly to H_2 receptors.

The benzisoxazol derivatives also distinguish themselves from haloperidol by their weak affinity for the haloperidol-sensitive σ sites. None of the compounds shows appreciable activity in the remaining tests, i.e., interactions occur only around micromolar concentrations or in many cases do not occur up to a concentration of 10 μM.

TABLE 2

Receptor binding and neurotransmitter uptake inhibition profile of ocaperidone, risperidone, and haloperidol

Tests were ordered according to increasing K_i values of ocaperidone; groups of neurotransmitter receptor subtypes were kept together, and within each group receptor subtypes were ordered according to increasing K_i values of ocaperidone. Values are mean ± standard deviation of the number of experiments in parentheses.

Receptor binding site	Radioligand	K_i values in nM					
		Ocaperidone		Risperidone		Haloperidol	
Serotonin $5HT_2$	[^{3}H]Ketanserin	0.14 ± 0.04	(4)	0.12 ± 0.02	(4)	25.1 ± 7.6	(4)
Serotonin $5HT_{1A}$	[^{3}H]8-hydroxydipropylaminotetralin	6.8 ± 0.9	(3)	270 ± 40	(3)	3,080 ± 1,680	(3)
Serotonin $5HT_{1D}$	[^{3}H]5HT	9.6 ± 1.3	(3)	52 ± 16	(4)	4,000 ± 1,000	(3)
Serotonin $5HT_{1C}$	[^{3}H]Mesulergine	28 ± 10	(3)	47 ± 6	(3)	NA[a]	
Serotonin $5HT_{1B}$	[^{3}H]5HT	540 ± 270	(3)	3,700 ± 1,700	(3)	NA	
Serotonin $5HT_3$	[^{3}H]GR65630	NA		NA		NA	
α_1-Adrenergic	[^{3}H]WB4101	0.46 ± 0.05	(3)	0.81 ± 0.14	(4)	10.9 ± 2.9	(4)
α_2-Adrenergic	[^{3}H]Clonidine	5.4 ± 1.1	(3)	7.3 ± 1.2	(4)	NA	
β_2-Adrenergic	[^{125}I]Iodocyanopindolol	460 ± 60	(2)	NA		NA	
β_1-Adrenergic	[^{125}I]Iodocyanopindolol	7,600 ± 1,500	(2)	NA		NA	
DA D_2	[^{3}H]Haloperidol	0.75 ± 0.08	(4)	3.0 ± 0.9	(4)	1.55 ± 0.47	(4)
DA D_1	[^{3}H]SCH23390	350 ± 90	(3)	620 ± 100	(3)	255 ± 41	(3)
Histamine H_1	[^{3}H]Pyrilamine	1.6 ± 0.2	(3)	2.10 ± 0.91	(3)	590 ± 50	(2)
Histamine H_2	[^{3}H]Tiotidine	430 ± 150	(4)	900 ± 300	(3)	5,100 ± 2,000	(2)
Haloperidol-sensitive σ sites	[^{3}H]Haloperidol	41 ± 16	(3)	800 ± 220	(3)	0.84 ± 0.1	(3)
Release site	[^{3}H]Ketanserin	57 ± 8	(3)	130 ± 30	(3)	380 ± 90	(3)
Na^+ channel	[^{3}H]Batrachotoxin	300 ± 70	(2)	3,850 ± 650	(3)	200 ± 70	(3)
Ca^{2+} channel	[^{3}H]Nitrendipine	3,200 ± 1,000	(3)	NA		1,110 ± 180	(2)
μ-Opiate	[^{3}H]Sufentanil	1,770 ± 120	(3)	NA		740 ± 120	(2)
Cholinergic muscarinic	[^{3}H]Dexetimide	1,950 ± 10	(3)	NA		NA	
TCP[b] *N*-methyl-D-aspartate sites	[^{3}H]TCP	NA		NA		4,500	(2)
Benzodiazepine	[^{3}H]Flunitrazepam	NA		NA		NA	
Cholecystekinin CCK-A	[^{3}H]CCK-8 (sulfated), propionylated	NA		NA		NA	
Cholecystekinin CCK-B	[^{3}H]CCK-8 (sulfated), propionylated	NA		NA		NA	
Neurotensin	[^{3}H]Neurotensin	NA		NA		NA	
Substance P	[^{3}H]Substance P	NA		NA		NA	
Leukotriene D_4	[^{3}H]Leukotriene D_4	NA		NA		NA	
Platelet-activating factor	[^{3}H]Platelet-activating factor	NA		NA		NA	
Thromboxane A_2	[^{3}H]SQ29548	NA		NA		NA	
Neurotransmitter uptake	**Radioligand**	**IC_{50}**					
		Ocaperidone		Risperidone		Haloperidol	
		nM					
Serotonin	[^{3}H]5HT	54 ± 4	(3)	540 ± 80	(4)	640 ± 70	(2)
NE	[^{3}H]NE	390 ± 70	(3)	2,540 ± 480	(4)	815 ± 260	(2)
DA	[^{3}H]DA	570 ± 70	(3)	4,740 ± 390	(3)	1,480 ± 270	(3)
γ-Aminobutyric acid	[^{3}H]γ-Aminobutyric acid	13,000 ± 5,000	(3)	NA		NA	

[a] NA, not active at the highest tested concentration, 10^{-5} M.
[b] TCP, *N*-[1-(2-thenyl)cyclohexyl]-3,4-piperidine.

In a similar way as reported previously for risperidone and haloperidol (2), we tested the *in vitro* dissociation rate of ocaperidone from the receptor sites for which it showed high affinity. Like risperidone, ocaperidone dissociated moderately slowly from $5HT_2$ and H_1 receptors (half-time of dissociation, $t_{1/2}$, of 30 and 40 min, respectively). It dissociated more rapidly from D_2 receptors and from α_1 and α_2 receptors ($t_{1/2}$, 10, 8, and 4 min, respectively).

The D_2 receptor interaction of the drugs was investigated in more detail in membrane preparations from the striatum, nucleus accumbens, and tuberculum olfactorium of the rat and of the 293 human embryonic kidney cells expressing the human D_2 receptor (29). K_D and B_{max} values, derived from saturation binding curves with 2′-[^{125}I]iodospiperone in the various tissues, are presented in Table 3. The experiments were performed in the presence (shown) and in the absence (not shown) of BW501, to occlude $5HT_2$ receptors. Identical results were obtained in the two conditions, indicating that $5HT_2$ receptors were not labeled in any of the tissues. The inhibition curves obtained with ocaperidone, risperidone, and haloperidol in the four tissues are shown in Fig. 2. In all the cases regular inhibition curves, fitting a one-site binding model, with Hill coefficients close to unity were obtained. Derived mean K_i values are presented in Table 3. The K_i values obtained in assays with 2′-[^{125}I]iodospiperone in dilute rat striatal preparations were 2–3.75 times lower than the ones measured in the more concentrated tissue preparations with [^{3}H]haloperidol as a ligand (Table 2); this is probably due to lower drug adsorption to the more dilute tissue. For each of the drugs the pIC_{50} values were the same in the four tissues (Fig. 2); the small nonsignificant differences in derived K_i values are due to variations, which are not significant, in the K_D value of 2′-[^{125}I]iodospiperone in the various tissues (Table 3).

TABLE 3

Characteristics of 2′-[^{125}I]Iodospiperone binding to DA D_2 receptors in membrane preparations of various tissues

Values are mean ± standard deviation of the numbers of experiments in parentheses.

	Rat striatum	Rat nucleus accumbens	Rat tuberculum olfactorium	Cloned human D_2 receptor in 293 human kidney cells
2′-[^{125}I]Iodospiperone				
K_D (nM)[a]	0.05 ± 0.01 (3)	0.07 ± 0.02 (2)	0.08 ± 0.01 (2)	0.09 ± 0.03 (2)
B_{max} (fmol/mg of tissue)[a]	20.2 ± 2.7	10.8 ± 1.2	5.3 ± 0.4	
B_{max} (fmol/mg of protein)[a]	870 ± 20	570 ± 27	280 ± 60	440 ± 130 (2)
Ocaperidone K_i (nM)	0.23 ± 0.03 (3)	0.29 ± 0.03 (3)	0.32 ± 0.05 (3)	0.40 ± 0.03 (3)
Risperidone K_i (nM)	1.4 ± 0.3 (3)	2.2 ± 0.9 (3)	2.0 ± 0.2 (3)	3.1 ± 0.1 (2)
Haloperidol K_i (nM)	0.5 ± 0.2 (2)	0.7 ± 0.2 (2)	1.1 ± 0.9 (2)	1.2 ± 0.5 (2)

[a] K_D and B_{max} values were derived from Scatchard plots, as described in Materials and Methods.

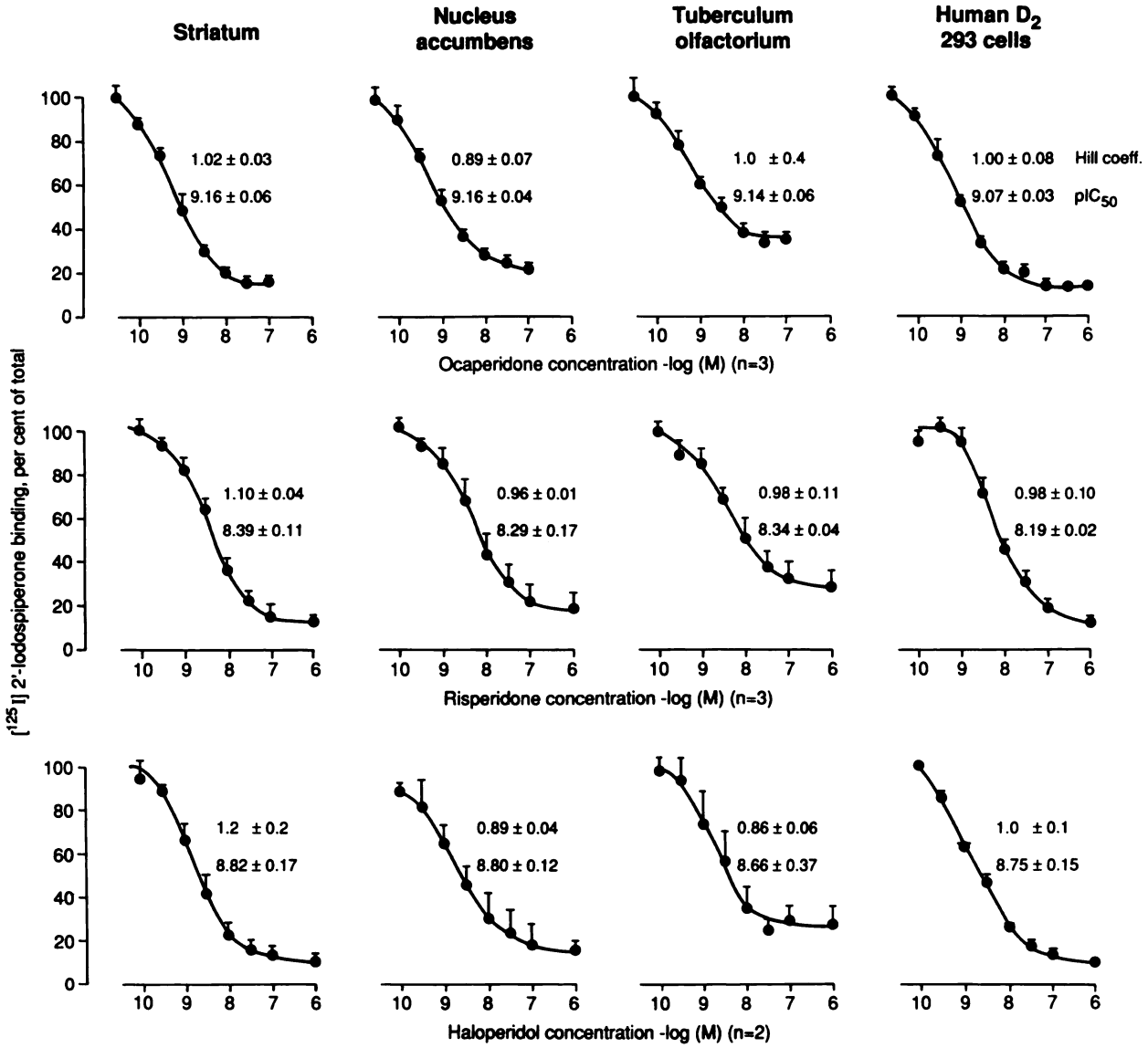


Fig. 2. Inhibition curves of ocaperidone, risperidone, and haloperidol for 2′-[^{125}I]iodospiperone (0.01 nM) binding to D_2 receptors in membrane preparations of the striatum, nucleus accumbens, and tuberculum olfactorium of the rat and in the 293 human kidney cell line transfected with the cloned human gene encoding the 443-amino acid-long D_2 receptor. Data are mean values ± standard deviations of *n* independent experiments, in duplicate. Hill coefficients and pIC_{50} values were obtained by nonlinear regression analysis of the separate curves and averaged.

***In vivo* receptor occupation.** The *in vivo* D_2 receptor occupancy in striatal and mesolimbic brain areas and $5HT_2$ receptor occupancy in the frontal cortex of rats were investigated using the *in vivo* radioligand binding technique, with [^{3}H]spiperone. Ocaperidone, risperidone, ritanserin, haloperidol, and clozapine were studied. The dose-response curves for *in vivo* inhibition of specific receptor labeling by [^{3}H]spiperone in the various brain areas are presented in Fig. 3.

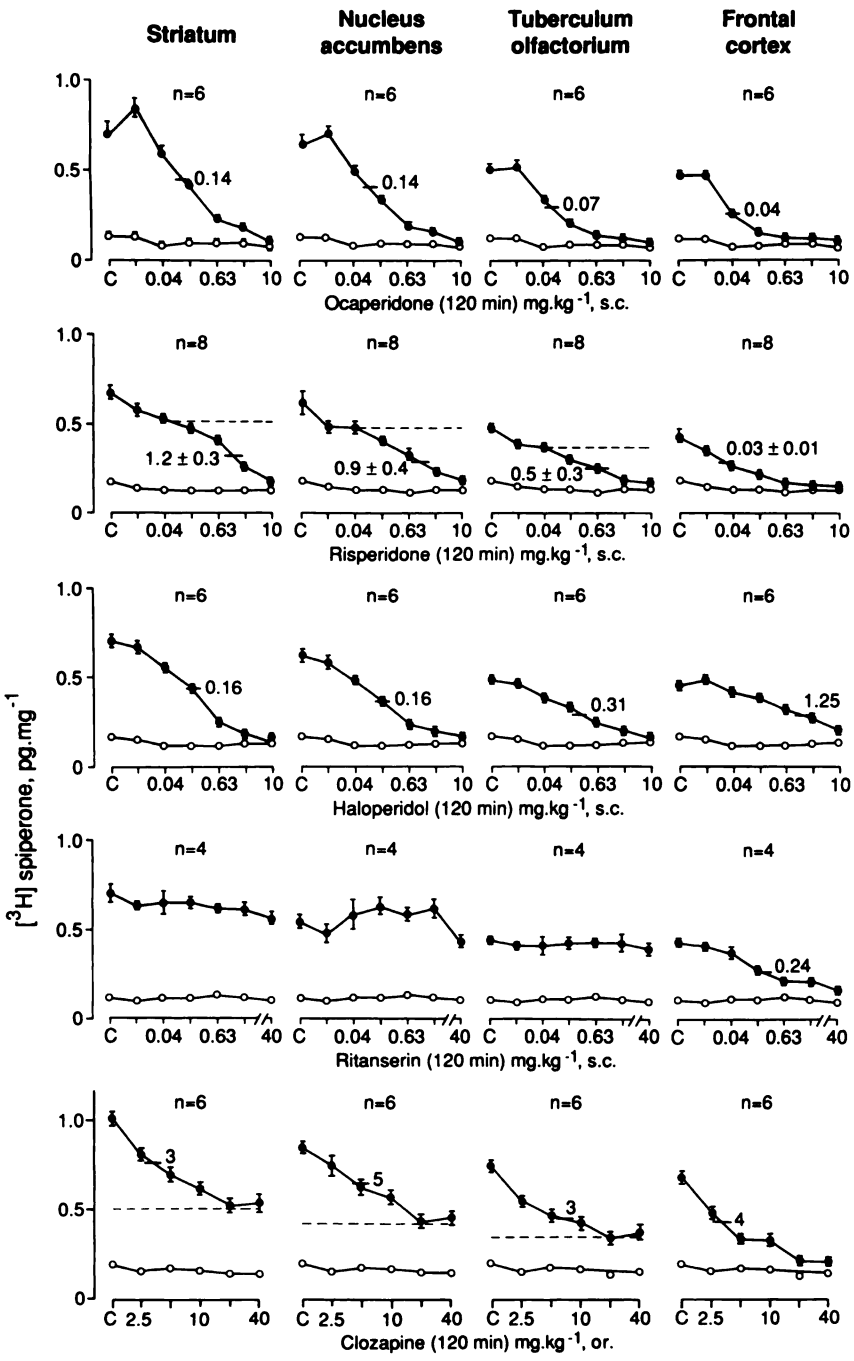


Fig. 3. *In vivo* binding of [^{3}H]spiperone in rat striatum, nucleus accumbens, tuberculum olfactorium, and frontal cortex. Labeling in the cerebellum (O) represents nonspecific binding. Prevention of *in vivo* labeling by ocaperidone, risperidone, haloperidol, ritanserin, and clozapine is shown. Dosages, route, and time of administration before sacrifice are indicated on the graphs. [^{3}H]Spiperone (5 μCi) was administered intravenously 1 hr before sacrifice. *Points* are mean values obtained in *n* series of animals. ED_{50} values indicated on the graphs were derived from the plots of mean values, except for risperidone, for which plots were analyzed separately for each series of animals. ED_{50} values for the second phase of the curve, starting at the *short horizontal line*, are indicated. For clozapine, ED_{50} values were estimated for the inhibited portion of the labeling (– – –).

Ocaperidone and risperidone inhibited labeling in the frontal cortex very potently (ED_{50} values of 0.04 and 0.03 mg/kg). Inhibition of labeling in this area was shown to represent $5HT_2$ receptor occupation (17). Ocaperidone and risperidone were considerably more potent than the other investigated drugs, 6-fold more potent than the $5HT_2$ antagonist ritanserin and 31-fold and 100-fold more potent, respectively, than the neuroleptics haloperidol and clozapine.

Ocaperidone and haloperidol revealed steep inhibition curves in the striatum and the nucleus accumbens. Both compounds were equipotent and their potency was the same in the two brain regions (ED_{50}, 0.14–0.16 mg/kg).

Risperidone revealed biphasic inhibition curves. Unfortunately, the number of data points that can be obtained by *in vivo* binding experiments to define these curves are limited, for practical reasons. With the available points, a statistically valid nonlinear regression analysis for two-site curve-fitting could not be performed. A graphical analysis of the curves from separate experiments allowed an estimation of the ED_{50} values for the upper and lower parts of the risperidone curves. The inhibition at low dosages is likely to represent $5HT_2$ receptor occupation (ED_{50} ~0.03 mg/kg) and that at higher dosages D_2 receptor occupation (ED_{50} ~1.0 mg/kg). At the latter site, risperidone was 6–7-fold less potent than ocaperidone and haloperidol. In the tuberculum olfactorium, the inhibition curve with ocaperidone was still steep, but the ED_{50} value was half that in the striatum. Haloperidol showed a shallow inhibition curve, with an ED_{50} double that in the striatum. Risperidone

again showed a biphasic inhibition curve, with an ED_{50} value for the lower part of half that in the striatum. Clozapine could only inhibit part of the labeling in the striatum, nucleus accumbens, and tuberculum olfactorium, with estimated ED_{50} values for the inhibited portion of about 4 mg/kg, i.e., equal to the ED_{50} value in the frontal cortex.

Ritanserin did not produce a significant inhibition of labeling in the striatum and the mesolimbic brain areas. Labeling in the cerebellum was not affected by any of the drugs. The inhibition curves of ocaperidone, risperidone, and haloperidol declined to cerebellar levels at the higher dosages. Cerebellar levels were taken as nonspecific labeling.

Effects on brain levels of monoamines and their metabolites. The levels of the monoamines NE, DA, and 5HT, the DA metabolites DOPAC and HVA, and the 5HT metabolite 5HIAA were measured in the striatum, nucleus accumbens, tuberculum olfactorium, frontal cortex, parietal cortex, and occipital cortex of control rats and of rats treated subcutaneously with various dosages of ocaperidone, risperidone, and haloperidol. The rats were killed by decapitation 2 hr after drug administration. The monoamine and metabolite levels, measured by HPLC, in tissues of control rats are summarized in Table 4. The levels, expressed as percentage of control values, in the drug-treated rats are shown in Fig. 4 for the various brain areas. The three drugs caused a marked rise in HVA and DOPAC in the dopaminergic brain areas, i.e., the striatum, the nucleus accumbens, and the tuberculum olfactorium. However, the shape of the dose-effect curves differed markedly for the drugs. With ocaperidone the curves reached a maximum plateau value, with risperidone the metabolite levels tended to increase continuously with increasing drug dose, and with haloperidol dome-shaped curves were obtained. In Table 5, dosages producing maximal HVA levels, the maximal levels of HVA and DOPAC, and the DA levels at the corresponding dose are reported. It is noted that with ocaperidone and haloperidol maximal HVA levels in the striatum and the nucleus accumbens were reached at the dose producing 50% D_2 receptor occupancy, whereas with risperidone 2–10 times this dose was required. Dosages for reaching maximal HVA levels in the tuberculum olfactorium were 4 times higher than in the striatum, and they were also higher than the apparent dose for 50% D_2 receptor occupancy in the tuberculum olfactorium. Maximal DA metabolite levels tended to be higher with risperidone, but because of large standard deviations the differences between the maximal levels were not statistically significant. Haloperidol caused, at higher dosages, a decrease in DA and 5HT levels. With ocaperidone and risperidone the decrease in monoamine levels was less consistent. In the frontal cortex, which contained very low levels of DA and barely detectable levels of DOPAC and HVA in control rats (Table 4), a rise in DOPAC levels was still apparent; with ocaperidone and risperidone, the maximal increase reached 250% and, with haloperidol, not even 200%. In the cortical areas, NE and 5HT levels appeared to decrease with the three drugs. The 5HT metabolite 5HIAA, although clearly detectable in all the examined brain areas in control animals, remained completely unchanged with all three drugs.

Discussion

***In vitro* receptor binding.** The extensive *in vitro* receptor binding profile of the drugs allows a detailed comparison between them. Ocaperidone and risperidone, which are very close structural congeners, share a number of properties, but they differ notably in affinity ratios for receptors and in the types of receptors occupied in the nanomolar concentration range. Both compounds primarily bind to and are equipotent at the $5HT_2$ receptor. In that regard, their K_i values are still 2 times lower than that of the $5HT_2$ antagonist ritanserin (2, 36), but they dissociate 5 times more rapidly from this receptor than does ritanserin. The three compounds show structural resemblance in the 2-methyl-3-ethyl-pyrimidine-4-one moiety of the molecule and can be considered as the most potent $5HT_2$ antagonists known thus far. Unlike ritanserin, ocaperidone and risperidone do not bind at nanomolar concentrations to the $5HT_{1C}$ receptor (18, 19). Receptors occupied with K_i values between 0.1 and 10 nM are, in order of increasing K_i value, for ocaperidone, $5HT_2 > \alpha_1 > D_2 > H_1 > \alpha_2 > 5HT_{1A} > 5\ HT_{1D}$, for risperidone, $5HT_2 > \alpha_1 > H_1 \geq D_2 > \alpha_2$ and, for haloperidol, $D_2 \sim$ haloperidol-sensitive σ sites. A notable difference between ocaperidone and risperidone is the $5HT_2/D_2$ receptor affinity ratio, which is 5.4 and 25, respectively, and the binding in the high nanomolar range of ocaperidone, but not of risperidone, to $5HT_{1A}$ and $5HT_{1D}$ receptor subtypes. The difference in profile between the benzisoxazol derivatives and haloperidol is obvious. The only common property is the nanomolar binding affinity for D_2 receptors; ocaperidone is 2 times more potent and risperidone 2 times less potent than haloperidol. Because D_2 receptor blockade is accepted to play a major role in the treatment of the positive symptoms of schizophrenia (see the introduction), one can expect that ocaperidone has the potential to be at least as effective as haloperidol for treating acute psychosis; risperidone may have a somewhat more moderate action. D_2 receptor blockade by itself also seems to be directly related to the induction of extrapyramidal side-effects. However, as explained above, very potent $5HT_2$ receptor blockade

TABLE 4

Content of monoamines and metabolites in various brain areas of control rats

Values are mean values ± standard deviations of 12 experiments.

	Control values					
	NE	DA	DOPAC	HVA	5HT	5HIAA
	pmol/mg of protein					
Striatum	3.8 ± 1.8	790 ± 270	80 ± 25	48 ± 17	18.2 ± 4.3	22.8 ± 6.5
Nucleus accumbens	25.3 ± 9.8	512 ± 118	70 ± 12	33 ± 8	32.8 ± 4.2	30.1 ± 8.1
Tuberculum olfactorium	22.8 ± 4.8	486 ± 63	51 ± 5	21.2 ± 3.9	70.0 ± 10	21.6 ± 3.4
Frontal cortex	14.2 ± 2.0	3.2 ± 1.0	0.65 ± 0.18	ND[a]	24.2 ± 6.5	8.5 ± 2.2
Parietal cortex	19.0 ± 3.3	ND	ND	ND	12.7 ± 1.5	8.7 ± 2.5
Occipital cortex	13.4 ± 2.6	ND	ND	ND	11.3 ± 2.4	6.0 ± 1.4

[a] ND, not detectable.

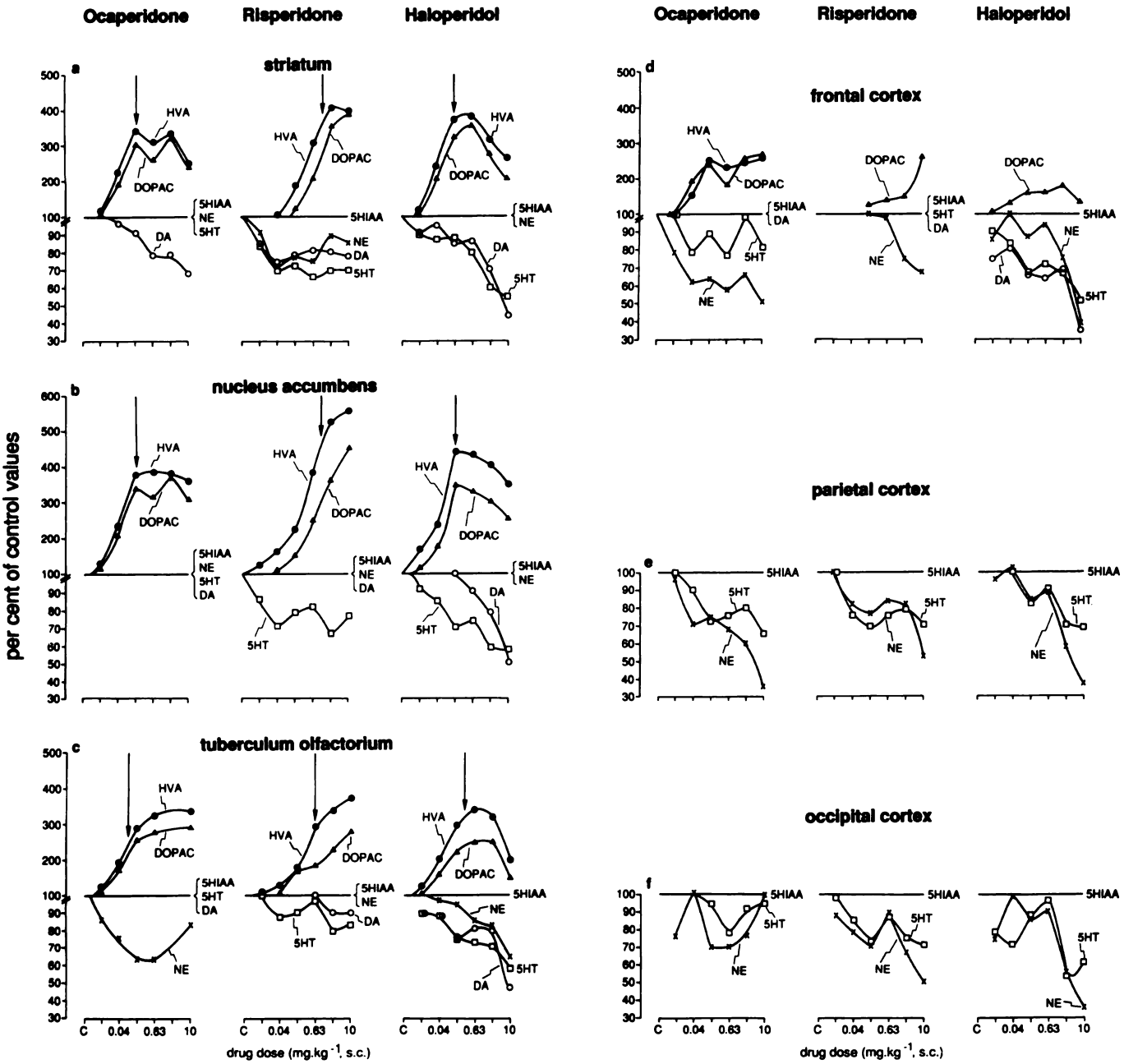


Fig. 4. Levels of monoamines and their metabolites in brain areas of rats, sacrificed 2 hr after subcutaneous treatment with various dosages of ocaperidone, risperidone, and haloperidol. Monoamine and metabolite levels were determined by HPLC in perchloric acid extracts of the indicated brain areas; levels are expressed as percentage of the control values shown in Table 4. Mean values of measurements in six animals (for risperidone and haloperidol) and three animals (for ocaperidone) are indicated, for clarity. To avoid overloading of the figure, *points* are only shown when Student *t* values for differences between treated and control animals were >2.8 (for $n = 3$) or >2.2 (for $n = 6$). Substances that were clearly detectable but remained unchanged are mentioned at the *line indicating the 100% level*. *Arrows*, dose producing 50% of occupancy of D_2 receptors in the various brain areas, measured in the *in vivo* binding experiment (see Fig. 3).

may help in attenuating these. Meltzer *et al.* (37, 38) claimed that, in a group of 38 neuroleptics classified as "atypical," i.e., with low propensity to induce extrapyramidal side-effects, or "typical," i.e., with higher side-effect liability, the $5HT_2/D_2$ ratio in pK_i values ($-\log K_i$) correlated with such a classification. Based on that analysis, risperidone, with a $5HT_2/D_2$ pK_i ratio of 1.16, would fall in the group with the lowest side-effect liability, whereas ocaperidone, with a ratio of 1.08, would fall in the middle group.

In addition to the attenuating effect of primary $5HT_2$ receptor blockade on the neuroleptic-induced motoric disturbances, clinical experiences with pipamperone, ritanserin, and risperidone have suggested that the very potent $5HT_2$ receptor blockade may be involved in the beneficial effect of these drugs on the negative symptoms of schizophrenia (3–6, 15, 16, 20). Hence, this therapeutic effect can also be expected with ocaperidone. The clinical correlates of blockade of certain receptor types, such as the D_2 and $5HT_2$ receptors, seem to become more and more established. Nevertheless, the entire binding profiles of the drugs, and in particular binding to those receptors that are occupied in the subnanomolar and nanomolar concentration range, are likely to contribute to the clinical picture of the

TABLE 5

Comparison of drug dosages producing 50% D_2 receptor occupancy and maximal HVA levels in various brain areas and comparison of maximal HVA and DOPAC levels produced by the drugs

Within a brain area, maximal HVA and DOPAC levels produced by the drugs were not statistically different. At the lowest dose where maximal HVA levels were reached, DA levels were not statistically different ($p > 0.05$) from control levels (Student t test, two-tailed).

	Dose producing		Maximal levels[a]		DA levels at the dose of maximal HVA levels[a]
	50% D_2 receptor occupancy	Maximal HVA levels	HVA	DOPAC	
	mg/kg		*% of control*		
Striatum					
Ocaperidone	0.14	0.16	342 ± 15 (3)	306 ± 15 (3)	91 ± 7 (3)
Risperidone	1.2	2.5	406 ± 66 (6)	355 ± 52 (6)	80 ± 13 (5)
Haloperidol	0.16	0.16	370 ± 111 (5)	322 ± 87 (5)	84 ± 22 (5)
Nucleus accumbens					
Ocaperidone	0.14	0.16	373 ± 24 (3)	339 ± 21 (3)	105 ± 9 (3)
Risperidone	0.9	10	554 ± 193 (6)	451 ± 125 (6)	107 ± 22 (6)
Haloperidol	0.16	0.16	443 ± 59 (6)	344 ± 51 (6)	101 ± 27 (5)
Tuberculum olfactorium					
Ocaperidone	0.07	0.63	323 ± 27 (3)	273 ± 32 (3)	110 ± 12 (3)
Risperidone	0.5	10	372 ± 73 (6)	283 ± 48 (6)	90 ± 10 (6)
Haloperidol	0.31	0.63	339 ± 70 (6)	247 ± 44 (6)	82 ± 17 (4)

[a] Values are mean values ± standard deviations of the number of experiments in parentheses.

drugs. A careful evaluation and comparison of profiles may help in eventually elucidating the role of certain receptors. Recurrent properties are interesting to establish correlations, but unique properties also deserve attention. In this respect, it was noted that ocaperidone and risperidone are the only drugs in the vast list of neuroleptics (8) with nanomolar binding affinity for α_2 receptors. In previous functional *in vitro* studies with risperidone, the drug was shown to have α_2 antagonistic properties, by which it enhanced norepinephrine release from brain slices (2). Although the *in vivo* consequence is not yet known, it has been hypothesized that α_2 antagonism may have antidepressant-like effects and, by concomitant α_2 receptor blockade, possible sedative effects attributed to α_1 receptor blockade could be overcome. It is tempting to speculate that this property, which is unique among neuroleptics, has a role in the reported appreciation of the drugs by patients (4).

***In vitro* interaction with D_2 receptors in various tissues.** Possible differences in pharmacological properties between DA receptors in different brain areas, particularly in the basal ganglia versus the mesolimbic dopaminergic brain areas, have long been a matter of debate. We now have new powerful tools available to scrutinize the receptor properties in *in vitro* binding studies. 2′-[^{125}I]Iodospiperone labeled exclusively D_2 receptors, with extremely high affinity (K_D value of 0.05–0.09 nM) (see Table 3). The advantageous properties of the radioligand allowed the use of very dilute tissue suspensions, which reduced the nonspecific binding to a minimum (see Fig. 2). Another new tool was the cloned human D_2 receptor expressed in mammalian cell lines. Two forms of the D_2 receptor, generated by differential splicing, exist in humans and rats. The receptors differ by a 29-amino acid-long part in the third cytoplasmic loop. The two forms appear not to differ in drug-binding properties, but the longer form is the predominant one in the brain and was demonstrated both on dopaminergic neurons (i.e., autoreceptor) and on postsynaptic neurons in the caudate putamen and in mesolimbic brain areas. For this study we used the long form (443 amino acids) of the cloned D_2 receptor, derived from a human pituitary cDNA library and expressed in human embryonic kidney 293 cells (29).

The binding affinities (K_D values) of the radioligand 2′-[^{125}I] iodospiperone for D_2 receptors in dilute membrane preparations of rat striatum, nucleus accumbens, and tuberculum olfactorium and of 293 cells expressing the human D_2 receptor did not differ significantly (see Table 3). Each of the drugs, ocaperidone, risperidone, and haloperidol, revealed exactly the same IC_{50} value in the four tissues (see Fig. 2). The potency ratios between the drugs were exactly the same as those found with the conventional assay using [^{3}H]haloperidol (Table 2). However, the absolute potency of the drug was apparently 2 times higher than in the conventional assay; this is likely to be due to the lesser loss of free drug out of solution in the very dilute tissue preparations used with 2′-[^{125}I]iodospiperone, compared with the 10–20-fold more concentrated tissue suspension used with [^{3}H]haloperidol. The most important finding here is that D_2 receptors in rat striatal and mesolimbic areas and human D_2 receptors show exactly the same binding properties for the presently studied DA D_2 antagonists; this finding was confirmed for a large series of drugs.[2]

Recently, two new subtypes of DA receptors, the D_3 receptor (39) and the D_4 receptor (40), which both appear to be related to the D_2 receptor, have been identified by gene cloning and expression. The D_3 receptor mRNA was particularly enriched in the islands of Calleja in the tuberculum olfactorium. Spiperone was reported to have high affinity for the cloned D_3 (K_i = 0.61 nM) (39) and the D_4 receptor (K_i = 0.08 nM) (40), as well as for the cloned D_2 receptor (K_i = 0.05–0.07 nM) (39, 40). In that respect, it could be possible that 2′-[^{125}I]iodospiperone could display a similar high affinity for the three DA receptor subtypes and that all three receptor subtypes would be labeled concomitantly by the ligand. Ocaperidone, risperidone, and haloperidol revealed regular sigmoidal inhibition curves, with slopes close to unity in all the examined tissues. Hence, these compounds did not distinguish any subtypes in the specific binding sites labeled by 2′-[^{125}I]iodospiperone. This could mean either that a homogeneous population of sites was labeled or that ocaperidone, risperidone, and haloperidol have a similar affinity for the three DA receptor subtypes. Because we did not

have cloned D_3 and D_4 receptors available, we could not check the various possibilities.

***In vivo* D_2 and $5HT_2$ receptor binding in rat brain.** An additional possibility for a differential action of drugs on mesolimbic versus striatal D_2 receptors would reside in a preferential penetration of certain compounds in mesolimbic brain areas. We investigated this by means of *in vivo* receptor binding, a technique by which a nonlabeled drug, followed by a radioligand, is administered to the living animals, allowing *in vivo* competition for receptor occupancy between the radioligand and the drug. The animal is then sacrificed and the radioactivity in dissected brain regions is measured. The inhibition of labeling by the administered nonlabeled drugs is a measure of receptor occupancy by that drug. We used intravenously injected [^{3}H]spiperone, which is known to label both D_2 and $5HT_2$ receptors (17, 30). The former are thought to be predominantly labeled in dopaminergic brain areas, particularly in the striatum and nucleus accumbens, the latter in the frontal cortex. Displaceable labeling was also detected in the tuberculum olfactorium. Besides ocaperidone, risperidone, and haloperidol, we investigated the selective $5HT_2$ antagonist ritanserin and the so-called atypical neuroleptic clozapine. The study of these drugs has allowed not only a comparison of their *in vivo* potency and regional action but also a further identification of the types of receptors labeled by [^{3}H]spiperone in the various brain areas.

Ocaperidone and risperidone, in agreement with their primary and extremely high affinity for $5HT_2$ receptors *in vitro*, inhibited most potently the labeling in the frontal cortex (ED_{50}, 0.03–0.04 mg/kg). They were by far the most potent compounds in the series. The *in vivo* potency ratios of the drugs, ocaperidone = risperidone > ritanserin > haloperidol > clozapine, strongly suggest that [^{3}H]spiperone indeed labeled exclusively $5HT_2$ receptors in the frontal cortex (Fig. 3). Only clozapine was relatively less active *in vivo*, compared with its *in vitro* binding affinity for $5HT_2$ receptors, which might be due to pharmacokinetic problems. In the striatum and nucleus accumbens, ocaperidone and haloperidol were the most potent agents (ED_{50}, 0.14–0.16 mg/kg) and their potency ratio versus the frontal cortex suggested that in the former areas the occupied receptors consisted mainly of D_2 receptors (Fig. 3). However, the biphasic inhibition curve of risperidone and the incomplete inhibition with clozapine (Fig. 3) indicated that some of the sites labeled with [^{3}H]spiperone in the striatum and the nucleus accumbens involved $5HT_2$ receptors. The upper part of the inhibition curve of risperidone probably reflects competition at $5HT_2$ receptors, because the dosages at which these sites were occupied (0.01–0.04 mg/kg) corresponded to inhibitory dosages in the frontal cortex (ED_{50}, 0.03 mg/kg). The inhibition seen with clozapine in the striatum and the nucleus accumbens probably involved, for the major part, $5HT_2$ receptors, because the ED_{50} value of the inhibited part corresponded to the ED_{50} in the frontal cortex.The *in vitro* measured affinity of clozapine for D_2 receptors is quite low, K_i = 150 nM verus K_i = 3.3 nM for $5HT_2$ receptors, i.e., a ratio of 45 (8, 41). In agreement with this wide *in vitro* potency difference, clozapine seemed to be unable to occupy D_2 receptors *in vivo* in the investigated dose range (up to 40 mg/kg). The second phase of the inhibition curve of risperidone in the striatum and nucleus accumbens probably represents occupation of D_2 receptors. With an ED_{50} value of about 1 mg/kg, the drug would cause a 7–6 times less potent *in vivo* D_2 receptor occupation than ocaperidone and haloperidol. Hence the potency difference between haloperidol and risperidone seemed to be somewhat greater *in vivo* than *in vitro*.

In tuberculum olfactorium, [^{3}H]spiperone apparently also labeled $5HT_2$ as well as D_2 receptors, with a higher $5HT_2$ fraction than in the striatum and the nucleus accumbens. This is in agreement with *in vitro* studies, which demonstrated that the tuberculum olfactorium contained 2 times more $5HT_2$ (41) than D_2 receptors (Table 3). In the present *in vivo* study, ocaperidone revealed a 2 times lower and haloperidol a 2 times higher ED_{50} value in the tuberculum olfactorium than in the striatum. Risperidone again showed a biphasic inhibition curve, with an ED_{50} value in the lower part 2 times lower than in the striatum. Clozapine showed only a partial inhibition, with an ED_{50} similar to that in the frontal cortex. Hence, ocaperidone and risperidone seemed to occupy D_2 receptors in the tuberculum olfactorium at lower dosages than in the striatum, in contrast to haloperidol, for which the reverse was observed. The tuberculum olfactorium is a typical mesolimbic brain area, and it is often alleged that preferential D_2 receptor blockade in the mesolimbic system would favor antipsychotic effects over extrapyramidal side-effects. The presently observed *in vivo* receptor occupancy with risperidone is likely to be due to the parent compound. The presence of risperidone metabolites in the brain after subcutaneous drug administration was investigated; after 2 hr, metabolites represented <10% of the parent compound in the frontal cortex and in the striatum, whereas in plasma nearly 60% of the drug was metabolized. Hence, risperidone metabolites appeared to penetrate poorly into the brain.[2,3] Data on ocaperidone metabolites are not yet available.

The *in vivo* studies have confirmed the predominant action of ocaperidone and risperidone on $5HT_2$ receptors, with the distinction between $5HT_2$ and D_2 receptor occupation being greater for risperidone than for ocaperidone. The virtual inability of clozapine for *in vivo* D_2 receptor occupation became apparent. It has become clear than, when [^{3}H]spiperone is used, findings could easily be misinterpreted, due to the presently demonstrated concomitant labeling by this ligand of $5HT_2$ and D_2 receptors in dopaminergic brain areas.

Effects on brain levels of monoamines and their metabolites. The investigation *ex vivo* of effects of administered drugs on monoamine and metabolite levels in the brain provides a neurochemical functional correlate of receptor blockade. Combining these data with the *in vitro* receptor binding profile and the *in vivo* receptor occupation of the drugs may reveal possible functional interactions between various receptors when blocked concomitantly. It has been amply documented that blockade of D_2 receptors induces a feedback enhancement of DA synthesis and release, resulting in higher DOPAC and HVA levels in dopaminergic brain areas (28). For drugs with less extrapyramidal side-effect liability, more preferential action in mesolimbic areas than in the striatum has been hypothesized but also refuted (24–27). In the present study we found that ocaperidone and risperidone, like haloperidol, readily increased the DOPAC and HVA levels in the striatum, nucleus accumbens, and tuberculum olfactorium. A slight but consistent rise of DOPAC in the frontal cortex, but not in other cortical areas, was also seen. The most conspicuous difference between

[2] J. Heykants, personal communication.
[3] J. Heykants, manuscript in preparation.

the three drugs was the different shape of the dose-effect curves (Fig. 4). Ocaperidone and haloperidol produced approximately the same maximal rise of HVA and DOPAC levels, which was reached at 0.16 mg/kg, i.e., the dose that produced 50% occupation of D_2 receptors in the striatum and the nucleus accumbens (Fig. 3; Table 5). With risperidone, a maximal rise in HVA and DOPAC was attained at the dose of 2.5–10 mg/kg, i.e., 2–10 times the ED_{50} value for *in vivo* D_2 receptor occupation. In the nucleus accumbens, the maximal HVA and DOPAC levels obtained with risperidone surpassed those of ocaperidone and haloperidol by 100–150% but, because of a large standard deviation, the effect was not statistically significant (Table 5). Recently, Saller *et al.* (14) suggested that $5HT_2$ receptor blockade further enhances the increase in DA turnover induced by D_2 receptor blockade. These investigators observed that a $5HT_2$ antagonist, ICI 169369 (10 mg/kg, intraperitoneally), further enhanced the DA metabolite rise induced by haloperidol (0.25 mg/kg, intraperitoneally) in the striatum but not in the tuberculum olfactorium. Unfortunately, effects were only investigated at one combination of dosages. Our findings revealed that, at dosages above 0.63 mg/kg subcutaneously, risperidone produced considerable higher levels of DA metabolites than did haloperidol. However, this is due to the fact that at the high dosages the effects of haloperidol had declined. The precise reason why the DA turnover is increased continuously with increasing dosages of risperidone and why effects of haloperidol go through a maximum and then decline cannot be derived with certainty from our experiments. Nevertheless, it could be that, because of the continuously enhanced DA turnover with risperidone, the functional antagonism between the increased released endogenous DA and the drug for D_2 receptor occupancy is more pronounced with risperidone than with haloperidol. Such functional antagonism could be less important in the tuberculum olfactorium, because risperidone produced a less steep rise in DA metabolite levels in that area than in the striatum and the nucleus accumbens. In line with the hypothesis of Saller *et al.* (14), the modulation of D_2 receptor blockade in the basal ganglia could provide a possible explanation for the lower cataleptogenic effects (42) and the reduced extrapyramidal side-effects with risperidone. On the other hand, the apparently relatively more potent D_2 receptor blockade by ocaperidone and risperidone in a typical mesolimbic area like the tuberculum olfactorium (Figs. 3 and 4C), and the clearer effect on the frontal cortical dopaminergic system, may explain the effectiveness of the drugs on the positive symptoms of schizophrenia, because these areas are thought to be important for psychosis.

Our findings further demonstrate that $5HT_2$ receptor blockade with ocaperidone and risperidone did not affect 5HIAA levels in any of the examined brain areas. Hence, in agreement with previous observations (36), $5HT_2$ receptor blockade apparently does not lead to a feedback increase in serotonin metabolism.

Effects on the monoamine levels themselves, which, when changed, were decreased, were rather variable with ocaperidone and risperidone in the various brain areas and were often not clearly dose related. Haloperidol appeared to produce, at the high dosages, a more consistent decrease of DA and serotonin levels in the striatum and the mesolimbic areas and of NE and serotonin levels in the tuberculum olfactorium and the cortical areas. The functional relevance of these high dose effects is unclear. Neither is there an obvious relation with a particular receptor occupation. Although σ receptor interaction is a property of haloperidol not shared by ocaperidone and risperidone, the role of that site, in this respect, remains to be investigated.

Conclusion

The integration of findings with the new benzisoxazol neuroleptics ocaperidone and risperidone and the classical reference drug haloperidol on the extensive *in vitro* receptor binding profile, the *in vivo* occupancy of $5HT_2$ and D_2 receptors in the frontal cortex, striatum, nucleus accumbens, and tuberculum olfactorium in rats, and the *ex vivo* measured drug effects on the levels of monoamines and their metabolites in these brain areas has provided further insight into the possible mechanism of action of the new drugs. Primary $5HT_2$ receptor blockade concomitant with D_2 receptor blockade apparently results in a relatively less avid D_2 receptor blockade, particularly in the basal ganglia. This may explain the reduced side-effect liability of the drugs. In this respect, it was demonstrated *in vitro* and *in vivo* that risperidone shows a wider difference between $5HT_2$ and D_2 receptor blockade than does ocaperidone. Very potent $5HT_2$ receptor blockade is also likely to play a role in the alleviation by $5HT_2$ antagonists of negative symptoms of schizophrenia and in their resocializing effects. The possibility that ocaperidone and risperidone, but not haloperidol, produce a relatively higher D_2 receptor blockade in the tuberculum olfactorium than in the basal ganglia and the apparent clearer effect of ocaperidone on the dopaminergic system in the frontal cortex probably underlie the effective antipsychotic action of the drugs. In addition, the property of the benzisoxazol neuroleptics to interact *in vitro*, at subnanomolar to nanomolar concentrations, with various other receptors should be kept in mind for the further interpretation of particular clinical features.

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