ABSTRACT
S 16924 showed a pattern of interaction at multiple (>20) native, rodent and cloned, human (h) monoaminergic receptors similar to that of clozapine and different to that of haloperidol. Notably, like clozapine, the affinity of S 16924 for hD2 and hD3 receptors was modest, and it showed 5-fold higher affinity for hD4 receptors. At each of these sites, using a \[^{[35S]}GTP\_S\] binding procedure, S 16924, clozapine and haloperidol behaved as antagonists. In distinction to haloperidol, S 16924 shared the marked affinity of clozapine for h5-HT\(_{1A}\) and h5-HT\(_{2C}\) receptors. However, an important difference to clozapine (and haloperidol) was the high affinity of S 16924 for h5-HT\(_{2A}\) receptors. At these sites, using a \[^{[35S]}GTP\_S\] binding model, both S 16924 and clozapine behaved as partial agonists, whereas haloperidol was inactive. In vivo, the agonist properties of S 16924 at 5-HT\(_{1A}\) autoreceptors were revealed by its ability to potently inhibit the firing of raphe-localized serotoninergic neurones, an action reversed by the selective 5-HT\(_{1A}\) receptor antagonist, WAY 100,635. In contrast, although S 16924 also suppressed 5-HT levels in the striatum and nucleus accumbens, DA levels therein were unaffected. Clozapine mimicked this selective increase in DA levels in the FCX as compared to striatum and accumbens. In contrast, haloperidol modestly increased DA levels in the FCX, striatum and accumbens to the same extent. In distinction to S 16924, clozapine and haloperidol exerted little influence upon 5-HT levels. Finally, the influence of S 16924 upon FCX levels of 5-HT, DA (and NAD) was attenuated by WAY 100,635. In conclusion, S 16924 possesses a profile of interaction at multiple monoaminergic receptors comparable to that of clozapine and distinct to that of haloperidol. In addition, S 16924 is a potent, partial agonist at 5-HT\(_{1A}\) receptors. Correspondingly, acute administration of S 16924 decreases cerebral serotoninergic transmission and selectively reinforces frontal-cortical as compared to subcortical dopaminergic transmission. In line with these actions, S 16924 shows a distinctive profile of activity in functional (behavioral) models of potential antipsychotic activity (companion paper).

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Classical neuroleptics, such as haloperidol, control the positive symptoms of schizophrenia (hallucinations, delusions, etc.) via the blockade of limbic D\(_2\) receptors targeted by hyperactive mesolimbic dopaminergic pathways (Holcomb et al., 1996; Kahn and Davis, 1995). However, neuroleptics are poorly effective against negative-cognitive symptoms, such as mutism and blunted affect. These symptoms reflect a disruption in the activity of mesocortical dopaminergic pathways and, more generally, a perturbation in the function of

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ABBREVIATIONS: AR, adrenergic; ANOVA, analysis of variance; CHO, Chinese hamster ovary; DA, dopamine; DOPA, dihydroxyphenylalanine; DOPAC, dihydroxyphenylacetic acid; DRN, dorsal raphe nucleus; EC\(_{50}\), effective concentration\(_{50}\); FCX, frontal cortex; GTP\_S, guanylyl 5\'-[\(\gamma\)-thio]-triphosphate; 5-HT, serotonin; 5-HTP, 5-hydroxytryptophane; HPLC, high-performance liquid chromatography; NAD, noradrenaline; VTA, ventral tegmental area.
the prefrontal cortex and FCX, commonly termed “hypofrontality” (Jentsch et al., 1997; Knable and Weinberger, 1997). Indeed, neuroleptics may exacerbate negative symptoms by blocking FCX-localized D2 receptors and provoking an extra-pyramidal syndrome. An additional disadvantage of neuroleptics is that a substantial population of patients do not respond satisfactorily to their administration (Kane and Freeman, 1994). Further, neuroleptics induce a pronounced hyperprolactinemia and associated endocrinological disorders by antagonism of tonically active D2 receptors on hypothalamic lactotrophs, and a marked extrapyramidal motor syndrome due to blockade of D2 receptors in the basal ganglia (Cunningham-Owens, 1996). Finally, long-term treatment with neuroleptics may ultimately result in the emergence of tardive dyskinesias, an irreversible motor problem likely related to striatal D2 receptor blockade, although its precise origin remains uncertain (Cunningham-Owens, 1996).

The above observations suggest that the improved treatment of schizophrenia requires drugs with characteristics different to those of typical neuroleptics and targeting sites other than, or in addition to, D2 receptors. In this respect, the dibenzodiazepine, clozapine, has attracted enormous interest inasmuch as this “atypical” antipsychotic manifests only modest affinity for D2 receptors yet is effective in a subpopulation of neuroleptic-resistant patients, improves negative symptomology, presents a benign extrapyramidal potential and does not elicit tardive dyskinesia (Kane and Freeman, 1994; Meltzer, 1995). An ongoing challenge is to identify the key receptorial interactions underlying the superior clinical profile of clozapine and, in this regard, numerous hypotheses have been formulated (Brunello et al., 1995; Kinon and Lieberman, 1996). These include: equilibrated antagonist activity at D1 and D2 receptors (Gerlach and Hansen, 1992); preferential antagonist activity at D2 vs. D4 receptors (Seeman et al., 1997) and pronounced antagonist activity at adrenergic (AR) receptors (Baldessarini et al., 1992). In addition, a convincing body of evidence points to the importance of 5-HT2A and, possibly, 5-HT2C receptors: these are concentrated in corticolimbic regions and the basal ganglia, are involved in the modulation of mood and motor behavior and modulate the activity of dopaminergic pathways (Brunello et al., 1995; Casey, 1996; Killand and Chiodo, 1996; Kennett et al., 1996; Roth and Meltzer, 1995; Schmidt and Fadayel, 2000).

**TABLE 1**
Radioligand binding conditions and affinities of S 16924 as compared to clozapine and haloperidol at multiple dopaminergic receptors

<table>
<thead>
<tr>
<th>Binding Site</th>
<th>Tissue</th>
<th>Radioligand (nM)</th>
<th>Nonspecific (µM)</th>
<th>Reference Ligand (Ki, nM)</th>
<th>S 16924 (Ki, nM)</th>
<th>Clozapine (Ki, nM)</th>
<th>Haloperidol (Ki, nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat D1</td>
<td>Striatum</td>
<td>[3H]SCH 23390 (0.2) (+Butaclamol (3))</td>
<td>SCH 23390 (0.3)</td>
<td>SCH 23390 (0.3)</td>
<td>189.0 ± 20.0</td>
<td>186.4 ± 6.2</td>
<td>47.8 ± 8.0</td>
</tr>
<tr>
<td>Human D1</td>
<td>L cells</td>
<td>[3H]SCH 23390 (1.0) SCH 23390 (10)</td>
<td>SCH 23390 (0.5)</td>
<td>SCH 23390 (0.5)</td>
<td>43.4 ± 0.4</td>
<td>90.0 ± 4.5</td>
<td>25.1 ± 5.3</td>
</tr>
<tr>
<td>Rat D2</td>
<td>Striatum</td>
<td>[3H]Haloperidol (2.0)</td>
<td>Haloperidol (1.0)</td>
<td>Haloperidol (1.0)</td>
<td>24.4 ± 7.2</td>
<td>231.0 ± 40.0</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Cloned rat D2</td>
<td>CHO cells [3H]iodosulpiride (2.0)</td>
<td>Schipirone (2.0)</td>
<td>Haloperidol (1.0)</td>
<td>Haloperidol (1.0)</td>
<td>5-HT (4.6)</td>
<td>57.3 ± 12.2</td>
<td>117.0 ± 37.0</td>
</tr>
<tr>
<td>Human D2</td>
<td>CHO cells</td>
<td>[3H]iodosulpiride (0.1)</td>
<td>Schipirone (2.0)</td>
<td>Schipirone (2.0)</td>
<td>46.3 ± 10.3</td>
<td>63.6 ± 1.5</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Cloned rat D2</td>
<td>CHO cells</td>
<td>[3H]iodosulpiride (2.0)</td>
<td>Schipirone (2.0)</td>
<td>Schipirone (2.0)</td>
<td>5-HT (2.0)</td>
<td>56.5 ± 6.0</td>
<td>226.0 ± 27.0</td>
</tr>
<tr>
<td>Human D2</td>
<td>CHO cells</td>
<td>[3H]iodosulpiride (1.0)</td>
<td>Schipirone (2.0)</td>
<td>Schipirone (2.0)</td>
<td>5-HT (2.0)</td>
<td>66.3 ± 17.2</td>
<td>289.0 ± 18.0</td>
</tr>
<tr>
<td>Human D1</td>
<td>CHO cells</td>
<td>[3H]iodosulpiride (2.0)</td>
<td>Schipirone (1.0)</td>
<td>Schipirone (1.0)</td>
<td>9.4 ± 1.3</td>
<td>32.6 ± 0.7</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>Human D1</td>
<td>GH cells</td>
<td>[3H]SCH 23390 (0.3) SCH 23390 (10)</td>
<td>SCH 23390 (0.4)</td>
<td>SCH 23390 (0.4)</td>
<td>34.0 ± 5.2</td>
<td>59.0 ± 4.7</td>
<td>28.0 ± 3.8</td>
</tr>
<tr>
<td>Rat DA reuptake</td>
<td>Rat striatum [3H]BTCP (0.5)</td>
<td>BTCP (10)</td>
<td>Nomifensine (43.3)</td>
<td>Nomifensine (43.3)</td>
<td>&gt;10000</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

All affinities are the means ± S.E.M. of ≥3 determinations. n.d., Not determined.

**TABLE 2**
Radioligand binding conditions and affinities of S 16924 as compared to clozapine and haloperidol at multiple serotonergic receptors

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Tissue</th>
<th>Radioligand (nM)</th>
<th>Nonspecific (µM)</th>
<th>Reference Ligand (Ki, nM)</th>
<th>S 16924 (Ki, nM)</th>
<th>Clozapine (Ki, nM)</th>
<th>Haloperidol (Ki, nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat 5-HT1A</td>
<td>Hippocampus</td>
<td>[3H]S-8-OH-DPAT (0.4)</td>
<td>5-HT (10)</td>
<td>5-HT (10)</td>
<td>3.8 ± 0.8</td>
<td>274.0 ± 45.0</td>
<td>3131 ± 675</td>
</tr>
<tr>
<td>Human 5-HT1A</td>
<td>CHO cells</td>
<td>[3H]S-8-OH-DPAT (0.4)</td>
<td>5-HT (10)</td>
<td>5-HT (10)</td>
<td>1.8 ± 0.2</td>
<td>154.0 ± 36.0</td>
<td>1927 ± 271</td>
</tr>
<tr>
<td>Guinea Pig 5-HT1</td>
<td>Striatum</td>
<td>[3H]HTS (2.0)</td>
<td>5-HT (10)</td>
<td>5-HT (10)</td>
<td>222.0 ± 44.0</td>
<td>6698 ± 2750</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>Rat 5-HT1A</td>
<td>Frontal cortex</td>
<td>[3H]S-8-OH-DPAT (1.0)</td>
<td>5-HT (10)</td>
<td>5-HT (10)</td>
<td>3.5 ± 0.8</td>
<td>23.1 ± 5.9</td>
<td>66.5 ± 7.7</td>
</tr>
<tr>
<td>Human 5-HT1A</td>
<td>CHO cells</td>
<td>[3H]S-8-OH-DPAT (0.5)</td>
<td>5-HT (10)</td>
<td>5-HT (10)</td>
<td>0.9 ± 0.4</td>
<td>1.7 ± 1.1</td>
<td>160.0 ± 78.0</td>
</tr>
<tr>
<td>Human 5-HT1A</td>
<td>CHO cells</td>
<td>[3H]S-8-OH-DPAT (1.0)</td>
<td>5-HT (10)</td>
<td>5-HT (10)</td>
<td>4.5 ± 2.1</td>
<td>1.6 ± 0.5</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Porcine 5-HT1C</td>
<td>CHO cells</td>
<td>[3H]S-8-OH-DPAT (1.0)</td>
<td>5-HT (10)</td>
<td>5-HT (10)</td>
<td>7.5 ± 1.1</td>
<td>6.0 ± 0.8</td>
<td>5743 ± 809</td>
</tr>
<tr>
<td>Guinea Pig 5-HT1</td>
<td>Striatum</td>
<td>[3H]S-8-OH-DPAT (0.1)</td>
<td>5-HT (10)</td>
<td>5-HT (10)</td>
<td>18.3 ± 4.1</td>
<td>45.0 ± 3.0</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>Human 5-HT1A</td>
<td>CHO cells</td>
<td>[3H]S-8-OH-DPAT (0.7)</td>
<td>5-HT (10)</td>
<td>5-HT (10)</td>
<td>1590 ± 90</td>
<td>&gt;10000</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>Cloned rat 5-HT1</td>
<td>CHO cells</td>
<td>[3H]S-8-OH-DPAT (1.0)</td>
<td>5-HT (10)</td>
<td>5-HT (10)</td>
<td>&gt;1000</td>
<td>&gt;10000</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>Rat 5-HT1A</td>
<td>HeK cell line</td>
<td>[3H]S-8-OH-DPAT (0.1)</td>
<td>5-HT (10)</td>
<td>5-HT (10)</td>
<td>27.0 ± 4.2</td>
<td>14.9 ± 3.1</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Cloned rat 5-HT1</td>
<td>CHO cells</td>
<td>[3H]S-8-OH-DPAT (2.0)</td>
<td>5-HT (10)</td>
<td>5-HT (10)</td>
<td>77.7 ± 9.8</td>
<td>54.4 ± 13.5</td>
<td>380.0 ± 106.0</td>
</tr>
</tbody>
</table>

All affinities are the means ± S.E.M. of ≥3 determinations. n.d., Not determined.
1995). Thus, clozapine has marked affinity for 5-HT2C receptors, blockade of which facilitates mesocortical dopaminergic transmission (Gobert et al., 1998 and unpublished observations; Kennett et al., 1997; Pessia et al., 1994). Further, a preferential blockade of 5-HT2A vs. D2 receptors by antipsychotic drugs, such as clozapine, has been convincingly correlated with a low propensity to elicit an extrapyramidal motor syndrome (Meltzer, 1995; Roth and Meltzer, 1995; Wadenberg, 1996) and changes in the levels of 5-HT2A receptors associated with a potentially fatal agranulocytosis in 1 to 2% of patients treated (Liu and Uetrecht, 1995).

In the light of the above observations, it would clearly be of interest to develop antipsychotic agents possessing the beneficial properties of clozapine yet lacking its disadvantages. In our efforts to identify such antipsychotic drugs, we have characterized a novel benzodiazepine, S 16924 (fig. 1). Herein, the receptorial profile of S 16924 is characterized, together with its modulation of dopaminergic, serotonergic and adrenergic transmission in cortical, limbic and striatal regions. In the following article, the putative antipsychotic as compared to extrapyramidal properties of S 16924 are documented.

### Methods

**Binding.** Competition binding studies were performed at multiple dopaminergic, serotonergic and AR receptor types, as well as at DA, 5-HT and NAD reuptake sites. Assay conditions are summarized in tables 1, 2 and 3 (see also Millan et al., 1995). Isotherms were analyzed by nonlinear regression, using the program “PRISM”

### Table 3

Radioligand binding conditions and affinities of S 16924 as compared to clozapine and haloperidol at multiple adrenergic receptors

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Tissue</th>
<th>Radioligand (nM)</th>
<th>Nonspecific (µM)</th>
<th>Reference Ligand (Ki, nM)</th>
<th>S 16924 (Ki, nM)</th>
<th>Clozapine (Ki, nM)</th>
<th>Haloperidol (Ki, nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat a1</td>
<td>Frontal cortex</td>
<td>[3H]prazosin (0.2)</td>
<td>Phentolamine (10)</td>
<td>Reference Ligand (Ki, nM)</td>
<td>S 16924 (Ki, nM)</td>
<td>Clozapine (Ki, nM)</td>
<td>Haloperidol (Ki, nM)</td>
</tr>
<tr>
<td>Rat a1A</td>
<td>Salivary gland</td>
<td>[3H]prazosin (0.4)</td>
<td>Phentolamine (10)</td>
<td>Reference Ligand (Ki, nM)</td>
<td>S 16924 (Ki, nM)</td>
<td>Clozapine (Ki, nM)</td>
<td>Haloperidol (Ki, nM)</td>
</tr>
<tr>
<td>Rat a1B</td>
<td>Liver</td>
<td>[3H]prazosin (0.04)</td>
<td>Phentolamine (10)</td>
<td>Reference Ligand (Ki, nM)</td>
<td>S 16924 (Ki, nM)</td>
<td>Clozapine (Ki, nM)</td>
<td>Haloperidol (Ki, nM)</td>
</tr>
<tr>
<td>Rat a2</td>
<td>Cortex</td>
<td>[3H]RX 821,002 (0.4)</td>
<td>Phentolamine (10)</td>
<td>Reference Ligand (Ki, nM)</td>
<td>S 16924 (Ki, nM)</td>
<td>Clozapine (Ki, nM)</td>
<td>Haloperidol (Ki, nM)</td>
</tr>
<tr>
<td>Human a2A</td>
<td>CHO cells</td>
<td>[3H]MK 912 (0.7)</td>
<td>Phentolamine (10)</td>
<td>Reference Ligand (Ki, nM)</td>
<td>S 16924 (Ki, nM)</td>
<td>Clozapine (Ki, nM)</td>
<td>Haloperidol (Ki, nM)</td>
</tr>
<tr>
<td>Human a2B</td>
<td>CHO cells</td>
<td>[3H]RX 821,002 (0.8)</td>
<td>Phentolamine (10)</td>
<td>Reference Ligand (Ki, nM)</td>
<td>S 16924 (Ki, nM)</td>
<td>Clozapine (Ki, nM)</td>
<td>Haloperidol (Ki, nM)</td>
</tr>
<tr>
<td>Human a2C</td>
<td>CHO cells</td>
<td>[3H]MK 912 (0.2)</td>
<td>Phentolamine (10)</td>
<td>Reference Ligand (Ki, nM)</td>
<td>S 16924 (Ki, nM)</td>
<td>Clozapine (Ki, nM)</td>
<td>Haloperidol (Ki, nM)</td>
</tr>
<tr>
<td>Human a3</td>
<td>CHO cells</td>
<td>[3H]RX 821,002 (2.0)</td>
<td>Phentolamine (10)</td>
<td>Reference Ligand (Ki, nM)</td>
<td>S 16924 (Ki, nM)</td>
<td>Clozapine (Ki, nM)</td>
<td>Haloperidol (Ki, nM)</td>
</tr>
<tr>
<td>Human ß1</td>
<td>Sf9 cells</td>
<td>[3H]CGP 12177 (0.15)</td>
<td>Alprazolam (50)</td>
<td>Reference Ligand (Ki, nM)</td>
<td>S 16924 (Ki, nM)</td>
<td>Clozapine (Ki, nM)</td>
<td>Haloperidol (Ki, nM)</td>
</tr>
<tr>
<td>Human ß2</td>
<td>Sf9 cells</td>
<td>[3H]CGP 12177 (0.15)</td>
<td>Alprazolam (50)</td>
<td>Reference Ligand (Ki, nM)</td>
<td>S 16924 (Ki, nM)</td>
<td>Clozapine (Ki, nM)</td>
<td>Haloperidol (Ki, nM)</td>
</tr>
</tbody>
</table>

All affinities are the means ± S.E.M. of 3 or 6 ± mean ± range of two determinations. n.d., Not determined.

Fig. 2. “Radar” representation of competition binding profiles of S 16924, clozapine and haloperidol at dopaminergic, serotonergic and adrenergic receptor types potentially implicated in the actions of clozapine and other antipsychotic agents: h, cloned, human; r, native rat and rc, cloned, rat. The distance from the center of the radar is proportional to the affinity (pK_i) of the compound at the receptor. pK_i (−log K_i) values were derived from results shown in tables 1, 2 and 3.
(Graphpad Software Inc., San Diego, CA) to yield Inhibitory Concentration (IC)\textsubscript{50} values. \(K_i\)s were derived from IC\textsubscript{50} values according to the Cheng-Prusoff equation: \(K_i = IC_{50}/(1 + L/K_d)\), where \(L\) is the concentration of radioligand and \(K_d\) is the dissociation constant of the radioligand.

**Measurement of agonist efficacy and antagonist potency at hD\textsubscript{2}, hD\textsubscript{3}, hD\textsubscript{4} and h5-HT\textsubscript{1A} receptors.** Receptor-linked G-protein activation at hD\textsubscript{2}, hD\textsubscript{3}, hD\textsubscript{4} and h5-HT\textsubscript{1A} receptors was determined by measuring the stimulation of \([\textsuperscript{35}S\text{-}]\text{GTP}\gamma\text{S}\) (1000 Ci/mmol; NEN, Les Ulis, France) binding as described in Newman-Tancredi et al. (1997). Briefly, CHO membranes (50 \(\mu\)g protein) expressing the respective receptors were incubated (20 min, 22°C) with agonists and/or antagonists in a buffer containing HEPES 20 mM (pH 7.4), GDP (3 \(\mu\)M), MgCl\(_2\) (3 mM for hD\textsubscript{2}, and hD\textsubscript{4}, 10 mM for hD\textsubscript{3}), NaCl (100 mM for hD\textsubscript{3} and hD\textsubscript{4}, 150 mM for hD\textsubscript{2}) and \([\textsuperscript{35}S\text{-}]\text{GTP}\gamma\text{S}\) (0.1 nM for hD\textsubscript{2} and hD\textsubscript{4}, 1 nM for hD\textsubscript{3}). Nonspecific binding was defined with \text{GTP}\gamma\text{S} (10 \(\mu\)M). Agonist efficacy was expressed relative to that of DA or 5-HT (100%) which were tested at maximally effective concentrations in each experiment. For antagonist studies, membranes were preincubated with antagonist and a single concentration of agonist for 30 min before the addition of \([\textsuperscript{35}S\text{-}]\text{GTP}\gamma\text{S}\). For concentration-response curves of the inhibition of DA-stimulated \([\textsuperscript{35}S\text{-}]\text{GTP}\gamma\text{S}\) binding, \(K_b\) values were calculated as described in Newman-Tancredi et al. (1997). Experiments were terminated by rapid filtration through Whatman GF/B filters using a Brandel cell harvester. Radioactivity retained on the filters was determined by liquid scintillation counting. Protein concentration was determined colorimetrically using a bicinchoninic acid assay kit (Sigma Chimie, St Quentin-Fallavier, France).

**In vivo studies.** Male Wistar rats (Iffa Credo, L’arbresle, France, 220–240 g body weight) were housed in sawdust-lined cages with free access to food and water. Laboratory temperature was 21 ± 1.0°C and humidity 60 ± 5%. There was a 12 hr/12 hr light-dark cycle with lights on at 7:30.

**Influence upon striatal DA and 5-HT turnover.** As described in detail previously (Gobert et al., 1995a), the influence of drugs upon striatal DA and 5-HT turnover in rats was evaluated by measuring the levels of the DA precursor, DOPA, and of the 5-HT precursor, 5-HTP in the striatum 60 min after s.c. injection of drugs and 30 min after injection of the decarboxylase inhibitor, NSD 1015 (100 mg/kg, s.c.). Tissues were homogenized in 500 \(\mu\)l of 0.1 M HClO\(_4\) containing 0.5% Na\(_2\)S\(_2\)O\(_5\) and 0.5% EDTA and centrifuged at 15,000 \(\times\) g for 15 min at 4°C. Supernatants were diluted in the mobile phase. HPLC
analysis followed by electrochemical detection was used for determination of tissue levels of DOPA and 5-HTP. The column characteristics and elution phases were as follows: column, Hypersil ODS (5 μm), C18, 150 × 4.6 mm maintained at 25°C; mobile phase, KH2PO4 (100 mM), EDTA (0.1 mM), sodium octylsulfonate (0.5 mM), methanol (5%) adjusted to pH 3.15 with PO4H3. The flow rate was 1 ml/min. Electrochemical detection was performed using a Waters M460 detector with a working potential of 850 mV against an Ag/AgCl reference. Levels of DOPA and 5-HTP were expressed relative to control (vehicle) values (−50%). Data were analyzed by ANOVA followed by Dunnett’s test.

**Influence on cerebral DA turnover.** As described in detail previously (Millan et al., 1995), the ratio of DOPAC to DA levels was determined in various cerebral tissues 30 min after s.c. administration of drugs. Levels were determined by HPLC/electrochemical detection as described above. DOPAC/DA ratios were expressed relative to control (vehicle) values (−50%). Data were analyzed by ANOVA followed by Dunnett’s test.

**Influence upon the electrical activity of dopaminergic neurones.** As previously described (Lejeune et al., 1997), rats were anesthetized with chloral hydrate (400 mg/kg, i.p.), the femoral vein was catheterized and rats were placed in a stereotaxic apparatus. A tungsten micro-electrode was lowered into the VTA. Dopaminergic neurones were identified as previously and baseline recording performed over 5 min. Drugs were dissolved in sterile water and injected i.v. in a volume of 0.5 ml/kg, followed by a 0.1 ml saline flush. Compounds were administered cumulatively i.v. at intervals of 2 to 5 min. In antagonist studies, they were administered (single dose) 2 min after the injection of apomorphine (0.031 mg/kg, i.v.). Data acquisition and analysis were performed using Spike 2 software (C.E.D., Cambridge, England) and results are expressed as firing rate (60-sec bins at time of peak drug action) as a percentage of baseline pre-injection values (−0%).

**Influence on the electrical activity of serotoninergic neurones.** For evaluation of the influence of drugs upon the activity of serotoninergic neurones of the DRN, an identical protocol was used as described above for dopaminergic neurones (Lejeune et al., 1994, 1997). Drugs were administered in cumulative doses i.v. at intervals of 2 to 5 min. In antagonist studies, drugs were administered at a
Results

Patterns of displacement. At each of the receptors presented in tables 1 to 3, S 16924, clozapine and haloperidol presented monophasic isotherms for displacement of the respective radioligands (slope factors not significantly differing from unity) (not shown). In figure 2, the overall receptor profiles of S 16924, haloperidol and clozapine at several key receptor types are depicted. It may be seen that the profiles of S 16924 and clozapine corresponded closely, whereas that of haloperidol was markedly different.

Affinities of S 16924, clozapine and haloperidol at multiple dopaminergic receptors (table 1). Whereas haloperidol displayed potent affinity at native rat and cloned hD2 receptors, S 16924 mimicked the modest affinity of clozapine at these sites. Similarly, the affinity of S 16924 and clozapine at cloned rat and hD3 receptors was modest in contrast to the marked affinity of haloperidol for these sites. Haloperidol manifested about 5-fold lower affinity for hD2 (the hD4.4 isoform) as compared to hD3 receptors whereas clozapine displayed a mild preference (about 2-fold) for hD3 sites. This preferential affinity for hD3 vs. hD2 sites was more pronounced for S 16924 (about 5-fold selectivity). Compared with D2 receptors, the affinity of haloperidol was markedly lower at both native D1 and cloned, hD1 receptors. In distinction, both S 16924 and clozapine presented comparable and modest affinity for native D1 and cloned hD1 receptors as compared with D2 receptors. Haloperidol, S 16924 and clozapine all showed similar affinities for cloned hD3 receptors as compared to hD2 receptors. The affinity of S 16924 at DA reuptake sites was negligible.

Affinities of S 16924, clozapine and haloperidol at multiple serotoninergic receptors (table 2). Haloperidol showed negligible affinity at native rat 5-HT1A and cloned h-5-HT1A receptors although the modest affinity of clozapine at these sites was similar to its affinity at D2 receptors (table 1). In contrast, S 16924 showed pronounced affinity at both rat 5-HT1A and h-5-HT1A receptors that was ~20-fold superior to its affinity at D2 sites. Haloperidol showed negligible affinity for 5-HT1B sites, at which clozapine displayed very weak and S 16924 only low affinity. The affinity of haloperidol at native 5-HT1A and cloned h-5-HT1A receptors was weak vs. its affinity at D2 sites, and haloperidol displayed negligible affinity for native 5-HT2C and cloned h-5-HT2C sites as well as for h-5-HT2B sites. In distinction, clozapine
and S 16924 both showed markedly higher affinity at native, 5-HTTα and cloned h5-HTTα vs. D2 receptors. Similarly, in contrast to haloperidol, both S 16924 and clozapine manifested marked affinity for native 5-HTTα and cloned h5-HTTα receptors. Interestingly, for all ligands, affinities were higher at cloned, h5-HTTα vs. native, porcine 5-HTTα sites, and this difference was significant (P < .05) for S 16924 and clozapine. Whether this observation reflects a species difference, or a difference between native, tissue vs. cloned, transfected receptors, remains to be elucidated. S 16924 and haloperidol also showed pronounced affinity for h5-HTTα sites. At 5-HT3 receptors, clozapine displayed modest affinity whereas neither haloperidol nor S 16924 displayed significant affinity. The affinity of all drugs for 5-HT3 and 5-HT5α sites was low. However, both S 16924 and clozapine, in contrast to haloperidol, showed significant affinity at 5-HT6 and 5-HT7 sites. S 16924 did not manifest significant affinity for 5-HT reuptake sites.

**Influence of S 16924, clozapine and haloperidol at multiple adrenergic receptors (table 3).** S 16924, clozapine and haloperidol all shared potent affinity for native α1-AR as well as α1A- and α1B-AR receptors. However, when expressed relative to their affinity at D2 receptors, S 16924 and clozapine, but not haloperidol, revealed a marked preference for α1A-, α1A- and α1B-AR sites in each case. S 16924 and clozapine showed modest affinity at both native α2A- and cloned hα2A-AR receptors. Further, they also showed modest affinity for cloned hα2A- and hα2C-AR receptors. The affinity of haloperidol for each of these α2-AR receptor types was negligible. S 16924 did not show significant affinity for β1- and β2-AR receptors or for NAD reuptake sites.

**Influence of S 16924 as compared to clozapine and haloperidol upon [3H]-GTPγS binding at hD2, hD3 and hD4 receptors.** Dopamine elicited a concentration-dependent increase in [3H]-GTPγS binding to cloned hD2, hD3 and hD4 receptors with Effective Concentration (EC50) values of 353 ± 52, 15.6 ± 3.9 and 109 ± 15 nM, respectively. In contrast, neither S 16924, clozapine nor haloperidol stimulated binding at these receptors (fig. 3 and not shown). In deed, they all behaved as antagonists at hD2, hD3 and hD4 receptors, concentration-dependently inhibiting the stimulation of [3H]-GTPγS binding induced by DA (3, 1 and 1 μM respectively) (fig. 3 and not shown). Kd values calculated for S 16924 were: hD2, 34.2 ± 3.7 nM; hD3, 79.8 ± 7.2 nM and hD4, 5.0 ± 1.8 nM. Kd values calculated for clozapine were: hD2, 71.7 ± 11.1 nM; hD3, 251 ± 80 and hD4, 48.4 ± 3.7 nM. Kd values calculated for haloperidol were: hD2, 0.58 ± 0.10; hD3, 28.8 ± 11.3 and hD4, 1.37 ± 0.18 nM.

**Influence of S 16924 as compared to clozapine and haloperidol upon [35S]-GTPγS binding at h5-HT1A receptors.** Serotonin concentration-dependently increased [35S]-GTPγS binding at h5-HT1A receptors with an EC50 of 16.8 ± 3.9 (fig. 4). Even at a very high concentration (10 μM), haloperidol failed to stimulate [35S]-GTPγS binding (not shown). However, clozapine (EC50 of 1740 ± 736 nM) stimulated binding to 43.8 ± 3.1% of levels attained with 5-HT (defined as 100%) (not shown). S 16924 stimulated [35S]-GTPγS binding by 54.1 ± 11.3%, but with considerably greater potency than clozapine: the EC50 of S 16924 was 11.3 ± 0.4 nM (fig. 4). S 16924-stimulated [35S]-GTPγS binding was inhibited by the selective 5-HT1A antagonist, WAY 100,635, with an IC50 of 3.18 ± 0.53 (fig. 4). WAY 100,635 was inactive alone (not shown).

**Influence of S 16924 as compared to clozapine and haloperidol upon cerebral turnover of DA and 5-HT.** As determined by the ratio of tissue levels of DA to those of its metabolite, DOPAC, haloperidol potently and markedly enhanced DA turnover in projection areas of mesocortical (FCX), mesolimbic (olfactory tubercles and nucleus accumbens) and nigrostriatal (striatum) pathways (fig. 5). In contrast, S 16924 and clozapine only weakly and less markedly increased DA synthesis in each of these regions (fig. 5). Similarly, on determination of levels of the DA precursor, DOPA, after pretreatment with the decarboxylase inhibitor, NSD 1015 (100 mg/kg, s.c.), haloperidol elicited a potent and pronounced induction in striatal DA synthesis whereas S 16924 and clozapine were only weakly active (fig. 5). Haloperidol failed to modify striatal levels of the 5-HT precursor,

![Influence of S 16924 as compared to clozapine and haloperidol on the electrical activity of dorsal raphe serotonergic neurons.](image-url)
5-HTP, an index of 5-HT synthesis, whereas striatal levels of 5-HTP were potently and markedly decreased by S 16924 and slightly depressed by clozapine (fig. 5).

**Influence of S 16924 as compared to clozapine and haloperidol upon the electrical activity of VTA-localized dopaminergic neurones.** The dopaminergic agonist, apomorphine (0.031 mg/kg, i.v.), markedly reduced the firing rate of dopaminergic neurones in the VTA (fig. 6). This action was dose-dependently inhibited by haloperidol and, less potently, by S 16924 and clozapine (fig. 6). ID_{50} (95% CLs) were as follows: 0.004 (0.002–0.006), 0.18 (0.12–0.19) and 0.22 (0.15–0.34), respectively. Administered alone, haloperidol and, less potently, clozapine and S 16924 slightly but dose-dependently and significantly increased firing rate (fig. 6).

**Influence of S 16924 as compared to clozapine and haloperidol upon the electrical activity of DRN-localized serotoninergic neurones.** S 16924 potently and dose-dependently inhibited the firing of DRN-localized serotoninergic neurones with an ID_{50} (95% CLs) of 0.02 (0.01–0.03) (fig. 7). Clozapine also inhibited DRN firing over a higher dose-range: ID_{50} (95% CLs) = 0.08 (0.02–0.3). Haloperidol was also effective, although only at high doses: ID_{50} (95% CLs) = 0.4 (0.2–0.9) (fig. 7). The inhibitory influence of S 16924 was blocked by WAY 100,635 and a further 5-HT_{1A} antagonist, (−)-tertatolol, neither of which significantly modified firing rate upon administration alone (fig. 7). The actions of clozapine and haloperidol were not affected by WAY 100,635 or (−)-tertatolol (fig. 7).

**Influence of S 16924 as compared to clozapine and haloperidol upon extracellular levels of DA, 5-HT and NAD in the FCX, nucleus accumbens and striatum of freely-moving rats.** Haloperidol (0.63) elicited a modest increase in dialysate levels of DA in the FCX and a similar, though more sustained, elevation in extracellular levels of DA levels in the nucleus accumbens and striatum (fig. 8). In contrast S 16924 (2.5) and clozapine (2.5) increased dialysate levels of DA in the FCX without markedly modifying those of DA in either the accumbens or striatum (fig. 8). This facilitatory influence of S 16924 on FCX levels of DA was ex-

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**Fig. 8.** Influence of S 16924 as compared to clozapine and haloperidol upon extracellular levels of DA in the frontal cortex as compared to the nucleus accumbens and striatum of freely-moving rats. Data are means ± S.E.M. N = 5 to 12 per value. They are expressed as a percentage of basal, preinjection values which were defined as 0%. These were 1.2 ± 0.09, 8.4 ± 1.3 and 12.9 ± 1.6 pg/20 min dialysate for DA in the frontal cortex, nucleus accumbens and striatum, respectively, in vehicle-treated animals. For comparison of individual values with the vehicle-treated group (open circles), ANOVA was performed over 40 to 180 min. Influence of S 16924: frontal cortex, F(1,16) = 56.5, P < .01; nucleus accumbens, F(1,18) = 0.2, P > .05 and striatum, F(1,16) = 9.9, P < .01. Influence of clozapine: frontal cortex, F(1,17) = 27.4, P < .01; nucleus accumbens, F(1,18) = 2.0, P > .05 and striatum, F(1,17) = 0.2, P > .05. Influence of haloperidol: frontal cortex, F(1,19) = 25.3, P < .01; nucleus accumbens, F(1,16) = 39.2, P < .01 and striatum, F(1,16) = 154.1, P < .01. Asterisks indicate significance of drug-treated groups to the vehicle-treated group. * P < .05.
pressed dose-dependently and, in parallel, it dose-dependently increased and decreased FCX levels of NAD and 5-HT, respectively (fig. 9). S 16924 also markedly decreased 5-HT levels in both accumbens and striatum (fig. 10). Clozapine also increased dialysate levels of NAD in the FCX (fig. 11) without significantly affecting levels of 5-HT either in this structure or in the accumbens, although it significantly decreased 5-HT levels in striatum (fig. 10). Haloperidol similarly provoked a modest increase in levels of NAD in FCX (fig. 11) without modifying levels of 5-HT in the FCX, accumbens or striatum (fig. 10). The influence of S 16924 upon FCX levels of DA and 5-HT was significantly inhibited by WAY 100,635 (fig. 12). In contrast, WAY 100,635 did not significantly modify the influence of clozapine upon FCX levels of DA, 5-HT or NAD (fig. 13).

**Discussion**

S 16924 displays a profile of interaction at multiple dopaminergic, serotonergic and adrenergic receptors similar to that of the “atypical” antipsychotic, clozapine and different to that of the neuroleptic, haloperidol. In particular, it shares the more marked activity of clozapine at hD4, 5-HT2A, 5-HT2C, and a1-AR as compared to D2 receptors. In addition, in contrast to both clozapine and haloperidol, S 16924 possesses potent, partial agonist properties at 5-HT1A receptors. This distinctive binding profile of S 16924 is reflected in vivo by its ability, upon acute administration, to inhibit serotoninergic transmission and to preferentially reinforce frontocortical vs. subcortical dopaminergic transmission.

**Antagonist properties at cloned hD2, hD3 and hD4 receptors.** S 16924 displayed, as with clozapine, only modest affinity at hD2 and hD3 receptors. In previous studies, clozapine has shown a mild, although variable and radioligand-dependent, preference for hD4 vs. hD2 receptors (Newman-Tancredi *et al.*., 1997; Seeman *et al.*, 1997), a finding confirmed herein, and S 16924 also displayed higher affinity at hD4 than hD2 receptors. Human D2, hD3 and hD4 receptors all couple via G proteins to (specific isoforms) of adenyl cyclase and other intracellular transduction systems (Levant, 1997; Newman-Tancredi *et al.*, 1997). Thus, activation and blockade of hD2, hD3 and hD4 sites can be evaluated by the binding of [35S]-GTPγS, which interacts with “activated” G proteins after their ligand-induced dissociation from the corresponding receptor. Using this approach, we recently demonstrated that clozapine and haloperidol behave as antagonists at hD4 receptors (Newman-Tancredi *et al.*, 1997) and S 16924 also behaved as a potent antagonist at hD4 sites. Our findings also show that S 16924, haloperidol and clozapine behave as antagonists at hD3 receptors. The antagonist properties of S 16924 at hD3 receptors are of significance inasmuch as blockade of postsynaptic D3 receptors in limbic structures, by counteracting the hyperactivity of mesolimbic dopaminergic pathways, may reduce the positive symptoms of schizophrenia (Kahn and Davis, 1995). Whether antagonism of postsynaptic D3 receptors, which are enriched in limbic tissue, is of importance to the actions of antipsychotic drugs is still under debate (Levant, 1997). The more pronounced activity of S 16924 at hD4 vs. hD2 receptors is of interest inasmuch as such a preference has been proposed to account for the superior antipsychotic profile of clozapine vs. haloperidol (Seeman *et al.*, 1997). This contention has, however, been challenged (Roth *et al.*, 1995) and putative alterations in levels of mRNA encoding D4 receptors in schizophrenics are controversial (Marzella *et al.*, 1997; Mulcrone and Kerwin, 1996; Seeman *et al.*, 1997). Further, the selective D4 receptor antagonist, L 745,870, was not effective in controlling psychosis in a clinical study (Kramer *et al.*, 1997). In addition, the preclinical profiles of L 745,870 and other selective D4 antagonists provide little evidence that antagonism of D4 receptors controls the positive symptoms of schizophrenia (Bristow *et al.*, 1997). Nevertheless, D4 receptor blockade may improve the cognitive-attentional...
Symptoms of schizophrenia (Tallman, 1997; Millan MJ and Dekeyne A, unpublished observations).

Interaction with D₁ and D₅ receptors. The similar affinity of S 16924 and clozapine at D₁ vs. D₂ receptors contrasts to the preference of haloperidol for the latter. This observation is of interest inasmuch as 1) a dysequilibrium in the activity of striatal populations of D₁ and D₂ receptors may contribute to extrapyramidal, side-effects and 2) actions at D₁ receptors may contribute to antipsychotic properties (Gerlach and Hansen, 1992) (companion paper). D₅ receptors present marked similarities to D₁ receptors, and clozapine and haloperidol possess similar affinity at hD₅ vs. hD₂ sites (Sunahara et al., 1991). Similarly, the modest affinity of S 16924 at hD₂ receptors was comparable to its affinity at hD₁ receptors. Although D₂ receptors are differentially localized to D₁ receptors, their functional significance remains unknown and certain actions ascribed to D₂ sites may actually be mediated by D₅ receptors (Bergson et al., 1995; Meador-Woodruff et al., 1996).

Antagonist actions at D₂ and D₃ receptors in vivo: modulation of cerebral DA synthesis. The activity of dopaminergic pathways is tonically inhibited by D₂ (and D₃) receptors localized on their dendrites and terminals and, possibly, by postsynaptic populations of D₃ sites acting via a feedback loop (Gobert et al., 1995b; Koeltzow et al., 1998; Tepper et al., 1997). Correspondingly, S 16924, clozapine and, more potently, haloperidol elevated DA synthesis in regions innervated by mesocortical (FCX), mesolimbic (olfactory tubercles and accumbens) and nigrostriatal projections (striatum) (Gobert et al., 1995a and b; Kahn and Davis, 1995). Interestingly, the magnitude of the increases in DA turnover evoked by S 16924 and clozapine were less marked than for haloperidol. One possible explanation is that elevations in DA turnover reflect inverse agonist actions at D₂ autoreceptors rather than blockade of tonic DA activity (Nilsso et al., 1996). However, as with haloperidol, clozapine possesses negative efficacy at hD₂ sites (Hall and Strange, 1997). An alternative explanation is that the serotoninergic

Figure 10. Influence of S 16924 as compared to clozapine and haloperidol upon extracellular levels of 5-HT in the frontal cortex as compared to the nucleus accumbens and striatum of freely-moving rats. Data are means ± S.E.M., N = 6 to 11 per value. They are expressed as a percentage of basal, pre-injection values which were defined as 0%. These were 0.80 ± 0.07, 0.65 ± 0.09 and 0.55 ± 0.07 pg/20 min dialysate for 5-HT in the frontal cortex, nucleus accumbens and striatum, respectively, in vehicle-treated animals. For comparison of individual values with the vehicle-treated group (open circles), ANOVA was performed over 40 to 180 min. Influence of S 16924, frontal cortex, F(1,9) = 31.3, P < .01; nucleus accumbens, F(1,14) = 43.5, P < .01 and striatum, F(1,11) = 29.6, P < .01. Influence of clozapine, frontal cortex, F(1,10) = 2.4, P > .05; nucleus accumbens, F(1,14) = 0.1, P > .05 and striatum, F(1,11) = 6.2, P < .05. Influence of haloperidol, frontal cortex, F(1,11) = 2.1, P > .05; nucleus accumbens, F(1,14) = 0.3, P > .05 and striatum, F(1,9) = 1.3, P > .05. Asterisks indicate the significance of drug-treated groups to vehicle-treated group. * P < .05.
Fig. 11. Influence of clozapine and haloperidol on extracellular levels of NAD in the frontal cortex of freely-moving rats. Data are means ± S.E.M. N = 6 to 11 per value. They are expressed as a percentage of basal, preinjection values that were defined as 0% (see legend to fig. 10). For comparison of individual values with the vehicle-treated group, ANOVA was performed over 40 to 180 min. Influence of clozapine, F(1,15) = 30.9, P < .01 and influence of haloperidol, F(1,17) = 16.7, P < .01. Asterisks indicate significance of drug-treated groups to vehicle-treated group.

and/or adrenergic actions of S 16924 and clozapine (see below) may intervene to moderate their influence upon DA synthesis. Irrespective of the underlying mechanisms, the finding that DA synthesis was little perturbed by S 16924 in the striatum is of importance inasmuch as extrapyramidal motor effects are correlated with an elevation of striatal DA synthesis (Lucas et al., 1997) (companion paper).

Partial agonist actions at 5-HT_{1A} receptors in vivo. Whereas S 16924 preferentially interacted at 5-HT_{1A} vs. D_{2} receptors, clozapine interacted with equivalent potency, and haloperidol interacted exclusively with D_{2} vs. 5-HT_{1A} receptors. In a [35S]GTPyS binding model, S 16924 behaved as a partial agonist with an efficacy equivalent to that of clozapine (Newman-Tancredi et al., 1996). This cellular model of 5-HT_{1A} receptor stimulation possesses a sensitivity comparable to that of postsynaptic 5-HT_{1A} receptors (Newman-Tancredi et al., 1997; Lejeune et al., 1997), at which S 16924 behaves as a partial agonist in vivo (companion paper). Inhibitory 5-HT_{1A} autoreceptors on serotoninergic cell bodies are more sensitive than their postsynaptic counterparts (Meller et al., 1990; Newman-Tancredi et al., 1997). Correspondingly, S 16924 markedly reduced striatal and acumens release of 5-HT and it reduced striatal turnover of 5-HT at doses substantially lower than those enhancing striatal DA turnover. These data, underpinned by the WAY 100,635-reversible inhibitory influence of S 16924 upon DRN firing rate and FCX dialysate levels of 5-HT, suggest that S 16924 acutely inhibits serotoninergic transmission via agonist ac-

tions at 5-HT_{1A} autoreceptors. This activity is of particular significance in several respects. First, stimulation of 5-HT_{1A} receptors facilitates the activity of mesocortical dopaminergic (and adrenergic) pathways (Lejeune et al., 1997; Millan et al., 1997) (see below). Second, an inhibition in 5-HT release is associated with a reduction in anxiety states (Coplan et al., 1995; Meller et al., 1990) and S 16924 possesses anxiolytic properties (Dekeyne A, and Millan MJ, unpublished observations). Third, activation of 5-HT_{1A} autoreceptors may counter the induction of extrapyramidal motor symptoms due to striatal D_{2} receptor blockade (Lucas et al., 1997) and S 16924 does not elicit catalepsy in rats (companion paper). In contrast to S 16924, clozapine only modestly inhibited striatal release and turnover of 5-HT and failed to modify dialysate levels of 5-HT in the accumbens or FCX. Further, the inhibitory influence of clozapine on DRN firing is mediated by its antagonist properties at α_{1}-AR receptors, a mechanism that may also intervene in the weak reduction of DRN firing by haloperidol (Lejeune et al., 1994).

Serotonin 5-HT_{2A} and 5-HT_{2C} receptors. A preferential blockade of 5-HT_{2A} vs. D_{2} receptors has been associated with a reduced propensity to elicit extrapyramidal side-effects and, possibly, an improved efficacy in the control of resistant patients and negative-cognitive symptoms (Roth and Meltzer, 1995; Schmidt and Fadayel, 1995). Thus, it is of significance that, in analogy to clozapine (Canton et al., 1994; Roth and Meltzer, 1995), S 16924 showed more pronounced affinity at 5-HT_{2A} than D_{2} receptors. Indeed, antagonism of 5-HT_{2A} receptors is an important, clozapine-like feature of the pharmacology of S 16924 (companion paper). The higher affinity of clozapine at 5-HT_{2C} vs. D_{2} sites (Canton et al., 1994; Roth and Meltzer, 1995) was similarly mimicked by S 16924. Although the significance of 5-HT_{2C} receptor blockade has been questioned as regards a reduced propensity to elicit extrapyramidal symptoms (Roth and Meltzer, 1995), there are several further, potentially important consequences of 5-HT_{2C} receptor blockade. First, antagonism of 5-HT_{2C} receptors markedly facilitates mesocortical dopaminergic transmission (Gobert et al., 1998; Kelland and Chiado, 1996; Pessia et al., 1994). Second, 5-HT_{2C} receptor antagonists display anxiolytic properties (Kennett et al., 1997). Third, based on studies of transgenic mice lacking 5-HT_{2C} receptors, it has been suggested that the weight gain provoked by antipsychotics reflects 5-HT_{2C} receptor blockade (Cunningham-Owens, 1996; Tecott et al., 1995). Nevertheless, certain antipsychotics, such as risperidone, elicit weight gain despite low affinity at 5-HT_{2C} receptors (Cunningham-Owens, 1996). Thus, other mechanisms, such as histamine_{1} receptor blockade, may also be involved (Cunningham-Owens, 1996). An interesting question concerns the functional significance of the combined blockade of 5-HT_{2A/2C} receptors and activation of 5-HT_{1A} receptors, as shown by S 16924. Serotoninergic transmission is inhibited by 5-HT_{1A} autoreceptors, and postsynaptic 5-HT_{1A} autoreceptors and 5-HT_{2A/2C} receptors exert an opposite influence on cellular transduction mechanisms, resulting in neuronal hyperpolarization and excitation, respectively. Thus, these properties may, as suggested previously, act synergistically (Millan et al., 1992) for example, in enhancing mesocortical dopaminergic transmission (see below). It would be of interest to perform long-term studies of the antipsychotic and other actions of the parallel activation and blockade of 5-HT_{1A} and 5-HT_{2A/2C} receptors, respectively.
5-HT_{6} and 5-HT_{7} receptors. S 16924 displayed significant affinity at 5-HT_{6} receptors and it has been proposed that an action of clozapine at these sites may contribute to its atypical profile (Monsma et al., 1993). Although Roth et al. (1994) suggested that relatively high affinity at 5-HT_{6} vs. D_{2} receptors may not be a distinguishing feature of "atypical" antipsychotics, the preferential corticolimbic localization of 5-HT_{6} receptors is of pertinence regarding the negative and cognitive-attentional symptoms of schizophrenia (Sleight et al., 1997). S 16924 also mimicked the high affinity of clozapine at 5-HT_{7} receptors. These are enriched in several limbic, cortical regions and have been implicated in depressive states, which can aggravate the negative symptoms of schizophrenia (Sleight et al., 1997; although see Gobbi et al., 1996). Moreover, sleep cycles are disrupted in schizophrenics and 5-HT_{7} receptors are concentrated in the suprachiasmatic nucleus wherein they fulfill an important role in controlling circadian rhythms (Lovenberg et al., 1993).

Interaction at α_{1}-AR receptors. A perturbation of adrenergic transmission is related to positive crises in schizophrenic patients and to an intensification of negative symptoms and the risk of relapse after treatment withdrawal (Mass et al., 1993). S 16924 mimicked the pronounced affinity of clozapine at α_{1}-ARs (as well as α_{1A}- and α_{1B}-ARs) (table 3), which are enriched in the thalamus, hippocampus, FCX and other structures implicated in the control of mood and in the pathophysiology of psychiatric disorders (Balldassarini et al., 1992). Several lines of evidence suggest that α_{1}-AR blockade may afford advantages in the treatment of schizophrenia. First, blockade of (limbic or cortical) α_{1}-AR receptors inhibits the induction of locomotion by psychostimulants (Blanc et al., 1994; Prinssen et al., 1994; Svensson et al., 1995). Second, coadministration of α_{1}-AR antagonists with haloperidol results in a clozapine-like, preferential inhibition of the activity of mesolimbic vs. nigrostriatal dopaminergic pathways (Lane et al., 1988). Third, blockade of thalamic α_{1}-AR receptors may improve the gating of sensory information to the cortex, a process that is defective in psychotic patients (Goldberg and Gold, 1995). Although peripheral α_{1}-AR blockade is associated with orthostatic hypotension, drug titration circumvents this effect, to which tolerance may develop (Cunningham-Owens, 1996).

Modulation of the electrical activity of VTA dopaminergic neurones. S 16924, clozapine and, more potently, haloperidol blocked suppression of the firing of VTA-localized dopaminergic neurones by apomorphine, consistent with their antagonist properties at D_{2} (and D_{3}) autoreceptors. In fact, they slightly enhanced firing rate when administered alone. This action of haloperidol may be attributed to the interruption of a tonic, inhibitory tone at D_{2} receptors (Gob-
Transmitter may improve the negative-cognitive symptoms correspondingly, a potentiation of mesocortical dopaminergic input to this region has been implicated (Andreasen et al., 1992). In this regard, a perturbation of dopaminergic pathways in the FCX fulfill an important role in mechanisms controlling vigilance and memory formation (Foote and Aston-Jones, 1995) suggesting that the potentiation in mesocortical adrenergic transmission by S 16924 may underlie its enhancement of FCX levels of DA. Such mechanisms may also intervene in the elevation in FCX dialysate levels of DA elicited herein by clozapine inasmuch as its actions were insensitive to WAY 100,635 (but see Rollema et al., 1997). The activity of mesocortical adrenergic neurones is subject to an inhibitory α2A-AR autoreceptor mediated-tone as well as a complex pattern of modulatory serotoninergic influence involving (indirect) facilitatory and inhibitory effects mediated via 5-HT1A and 5-HT2A/5-HT2C receptors, respectively (Gobert et al., 1998; Haddjeri et al., 1997; Millan et al., 1997). Inasmuch as the increase in FCX levels of NAD elicited by S 16924 and clozapine was not markedly attenuated by WAY 100,635, activation of 5-HT1A receptors may play a less important role in these actions than blockade of 5-HT2C (or α2-AR) receptors. In any case, adrenergic pathways in the FCX fulfill an important role in mechanisms controlling vigilance and memory formation (Foote and Aston-Jones, 1995) suggesting that the potentiation in mesocortical adrenergic transmission by S 16924 may be of use in improving cognitive-attentional performance.

Conclusions. For antipsychotic agents, it is their global pattern of interaction at multiple monoaminergic receptor types, and the relationship between the affinity at specific receptor types to that at D2 receptors, which determines their functional activity in vivo (see fig. 2). In this respect, S 16924 displays a profile of action that differs markedly to that of haloperidol and closely resembles that of clozapine, despite their chemical distinctiveness. In addition, the partial agonist properties of S 16924 at 5-HT1A receptors are more pronounced than those of clozapine. This distinctive component of activity underlies the acute inhibition of serotoninergic transmission by S 16924, fulfills an important role in its selective facilitatory influence on mesocortical dopaminergic
transmission, and, as described in the companion paper, contributes to its distinctive pattern of functional actions in experimental models of potential antipsychotic and extrapyramidal-ramidal activity.

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