A common action of clozapine, haloperidol, and remoxipride on D1- and D2-dopaminergic receptors in the primate cerebral cortex

(neuroleptic drugs/schizophrenia/association cortex/receptor autoradiography/receptor regulation)

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ABSTRACT The potencies of the major neuroleptics used in the treatment of schizophrenia, including haloperidol and remoxipride, correlate with their ability to bind D2-dopaminergic receptors in subcortical structures. On the other hand, the neuroleptic clozapine has a low affinity for these sites, and the pharmacological basis of its beneficial action is less clear. We have found that chronic treatment with clozapine, haloperidol, and remoxipride up-regulates D1 receptors in specific cortical areas of the rhesus monkey frontal, parietal, temporal, and occipital lobes. Of particular interest, all three neuroleptics down-regulated D1 receptors in prefrontal and temporal association regions—the two areas most often associated with schizophrenia. This latter finding raises the possibility that down-regulation of D1 receptors in prefrontal and temporal cortex may be an important component of the therapeutic response to neuroleptic drugs. Further, the common effects of three neuroleptics with different pharmacological profiles in the cerebral cortex is consistent with the idea that this structure is a major therapeutic target in the treatment of schizophrenia.

The therapeutic effect of neuroleptics has generally been associated with their binding to the D2 class of dopaminergic receptors in the brain (1). However, the beneficial actions of clozapine have not been satisfactorily explained, given that this drug does not interact with D2 sites in most regions examined (2, 3). Numerous previous studies of the effects of prolonged neuroleptic treatment on dopaminergic receptors have focused on subcortical structures (3-8), while the regulation of cerebral cortical dopaminergic sites by similar treatment has received little attention. Such data are particularly important in view of the mounting evidence for the involvement of the cortical dopaminergic system in schizophrenia (for reviews see refs. 9 and 10). To gain further insight into the mechanism of action of clozapine, we compared its effect on dopamine receptors in the cerebral cortex of rhesus monkeys with those of two representative neuroleptics, haloperidol and remoxipride. Both of these drugs display a high affinity for the D2-dopaminergic receptor sites in the neostriatum and nucleus accumbens, though remoxipride is more selective (11).

MATERIALS AND METHODS

Sixteen rhesus monkeys (Macaca mulatta), 5–7 years of age, were divided into four groups of equal size. Group H received haloperidol (0.2 mg/kg per day), group C1 received clozapine (5.2 mg/kg per day), and group R received remoxipride (3.7 mg/kg per day). These doses fall within the recommended range for therapeutic effects in schizophrenic patients (12, 13). The drugs were given orally (in fruit treatments) twice a day for 6 months to approximate the maintenance of medication in clinical practice (14). A control group (group CO) received only fruit treats. Five days after final administration of drugs, the animals were sacrificed, and cortical dopamine receptors were assayed by quantitative receptor autoradiography. The cortical regions examined are shown in Fig. 1A. They include prefrontal association (cytoarchitectonic areas 46 and 9 of Walker (15)), temporal association [area 21 of Brodmann (16)], primary motor [area 4 of Brodmann (16)], somatosensory [areas 1, 2, and 3 of Brodmann (16)], and primary visual [area 17 of Brodmann (16)] cortex.

D1-dopaminergic sites were labeled with the antagonist [125I]SCH23390 (17, 18). An example of [125I]SCH23390 binding in the prefrontal cortex is shown in Fig. 1B. Tissue sections were first preincubated for 20 min at room temperature in 50 mM Tris-HCl buffer, pH 7.4. They were then incubated with 0.1–10 nM [125I]SCH23390 for 90 min at room temperature in 50 mM Tris-HCl buffer, pH 7.4, containing 120 mM NaCl, 5 mM KCl, 2 mM CaCl2, 1 mM MgCl2, and 1 μM mianserin. The latter was added to block binding to 5-HT2, 5-HT1C, and α2 sites. After incubation, the tissue was rinsed twice (10 min each) in ice-cold 50 mM Tris-HCl buffer, pH 7.4. Nonspecific binding was determined in the presence of 1 μM cis-flupenthixol.

D2-dopaminergic receptors, illustrated in the prefrontal cortex (Fig. 1C), were labeled with the antagonist [125I]epidepride (18). Sections were preincubated as described for [125I]SCH23390 binding and then incubated for 45 min at room temperature with 0.1–3.0 nM [125I]epidepride in 50 mM Tris-HCl buffer, pH 7.4, containing 120 mM NaCl, 5 mM KCl, 2 mM CaCl2, 1 mM MgCl2, 0.1% ascorbic acid, and 0.2 μM idazoxan to prevent labeling of α2 sites. After incubation, the sections were rinsed as described for [125I]SCH23390 binding. Nonspecific labeling was determined in the presence of 10 μM (+)-butaclamol.

At the end of the labeling assays, all tissue sections were dipped in distilled water and apposed to 1-H-sensitive Ultrascan X-ray film (Amersham) for 6 weeks ([125I]SCH23390) or 1 week ([125I]epidepride). After development for autoradiography, the tissue sections were stained with cresyl violet to allow analysis of the cytoarchitecture. For all animals, binding assays were repeated twice. At least five tissue sections were processed for every concentration of ligand, three for total binding and two for nonspecific binding.

The autoradiograms were analyzed with a BDS computer system (Biological Detection Systems, Pittsburgh; ref. 17) and the statistical analysis of saturation binding was performed with the nonlinear curve-fitting computer programs KINETIC/EDBA/LIGAND/LOWRY from Elsevier-Biosoft (Cambridge, U.K.). The analysis is based on specific radiolabeling in tissue exposed to seven different concentrations of radioactive ligand in incubating solutions. This number of data points is sufficient to allow a relatively accurate estimation of Bmax (maximal binding) and Kd (steady-state dissociation constant) values for a one-site receptor model (17). Examples of saturation and Scatchard plots of [125I]SCH23390 and [125I]-

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epidepride are presented in Fig. 2. B_max values for control and each of the treated groups were compared with two-tailed Student t tests. The effects of neuroleptic treatments on K_d values were evaluated with a one-way ANOVA.

RESULTS AND DISCUSSION

Saturation analysis of [125]epidepride binding showed that all three neuroleptics produced statistically significant increases (47–52%) in the density of D_2-dopaminergic receptor sites in all layers of the temporal association, primary motor, somatosensory, and primary visual cortical regions (Fig. 3A). D_2 receptor sites were also increased in the prefrontal cortex but by a smaller percentage (11–18%) which did not reach statistical significance (Fig. 3A). None of the changes in receptor density were accompanied by changes in the affinity for [125]epidepride (Table 1). Our findings with haloperidol and remoxipride are in line with the widely reported D_2 up-regulation by these drugs in subcortical structures (3–7). However, the up-regulation of cortical D_2 receptors by clozapine in widespread areas of the cerebral cortex differs from the apparent lack of such effect of clozapine on these regions.

Table 1. Changes in apparent affinity values for binding of radioligands in the cortex of monkeys chronically treated with neuroleptics

<table>
<thead>
<tr>
<th>Radioligand</th>
<th>Cortical area</th>
<th>Control</th>
<th>Haloperidol</th>
<th>Clozapine</th>
<th>Remoxipride</th>
<th>P by one-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>[125]Epidepride</td>
<td>Prefrontal</td>
<td>10.17 ± 0.62</td>
<td>10.12 ± 0.43</td>
<td>10.05 ± 0.67</td>
<td>10.15 ± 0.83</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Temporal</td>
<td>10.21 ± 0.43</td>
<td>10.08 ± 0.99</td>
<td>9.91 ± 0.97</td>
<td>10.32 ± 0.76</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Motor</td>
<td>10.33 ± 1.01</td>
<td>9.97 ± 0.96</td>
<td>10.02 ± 0.60</td>
<td>10.37 ± 0.83</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Somatosensory</td>
<td>10.25 ± 0.31</td>
<td>9.86 ± 0.81</td>
<td>9.77 ± 0.99</td>
<td>10.17 ± 0.69</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Visual</td>
<td>10.18 ± 0.54</td>
<td>10.06 ± 1.11</td>
<td>9.86 ± 0.88</td>
<td>9.99 ± 0.71</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Prefrontal</td>
<td>9.31 ± 0.75</td>
<td>9.97 ± 0.62</td>
<td>9.78 ± 0.93</td>
<td>8.03 ± 1.20</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Temporal</td>
<td>9.86 ± 0.88</td>
<td>8.13 ± 1.13</td>
<td>9.88 ± 1.00</td>
<td>9.74 ± 0.78</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Motor</td>
<td>9.93 ± 0.89</td>
<td>9.85 ± 0.81</td>
<td>9.99 ± 0.70</td>
<td>8.05 ± 0.97</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Somatosensory</td>
<td>8.02 ± 1.10</td>
<td>8.05 ± 0.96</td>
<td>9.85 ± 0.62</td>
<td>9.91 ± 1.11</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Visual</td>
<td>9.65 ± 0.77</td>
<td>8.12 ± 1.10</td>
<td>9.51 ± 0.83</td>
<td>9.33 ± 1.26</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Since apparent affinities of the ligands have log-normal distributions, the data are presented and analyzed as -log K_d.
Fig. 3. Bar graphs representing changes in the density of D2-specific \[^{125}\text{I} \text{Epidepride}\] (A) and D1-specific \[^{3}\text{H} \text{SCH23390}\] (B) binding in response to chronic haloperidol, clozapine, and remoxipride treatment in different cortical regions of rhesus monkey. The data for prefrontal areas 9 and 12 were similar to those for area 46, and the data for somatosensory areas 2 and 3 were similar to those for area 1. The \(B_{\text{max}}\) value for each column is an average of four animals. Error bars are \(\pm\) SEM. Statistically significant differences between control and treated groups (two-tailed Student \(t\) test; \(P < 0.05\)) are marked by asterisks. Note that all three neuroleptics up-regulate the \[^{125}\text{I} \text{Epidepride}\] binding in most cortical areas. \[^{3}\text{H} \text{SCH23390}\] binding is down-regulated only in the prefrontal and temporal regions.

Fig. 4. Bar graph representing changes in the density of D1-specific \[^{3}\text{H} \text{SCH23390}\] binding in response to chronic clozapine treatment in individual layers of area 46 in the prefrontal cortex. In all cases, \(B_{\text{max}}\) values are averaged for four animals. Error bars are \(\pm\) SEM. Receptor densities in each layer differ from control values (two-tailed Student \(t\) test; \(P < 0.05\)) are marked by asterisks. Note that clozapine significantly down-regulates \[^{3}\text{H} \text{SCH23390}\] binding in all layers of the prefrontal cortex. Haloperidol and remoxipride have similar effects on \[^{3}\text{H} \text{SCH23390}\] receptors in all layers of the same cortical area.
effect of chronic neuroleptic treatment on cortical dopami-
nergic release after treatments as prolonged as that used in
the present study is not presently known. It is possible,
therefore, that very prolonged exposure to neuroleptics may
produce depolarization block of the cortical dopaminergic
innervation (25). Depolarization block can also down-
regulate D1 receptors (26), although this down-regulation
would be related to the elimination of phasic dopamine
release (27). Alternatively, the decrease in D1 receptor den-
sity may be secondary to a neuroleptic-induced increase in
the level of responsiveness of D2-mediated adenylate cyclase
to dopamine (6). As a strong interaction between D1 and D2
second messenger systems is well established (28, 29), it is
conceivable that an increase in the sensitivity of D2-
associated adenylate cyclase would be accompanied by an
increase in the sensitivity of the D1-coupled enzyme which,
in turn, would downregulate D1 receptors (8, 30).

Our findings that haloperidol, remoxipride, and clozapine
have virtually identical effects in all cortical areas examined
may provide the missing link in understanding the therapeutic
actions of these very different neuroleptic drugs. It seems
particularly relevant that the D1 down-regulation was found
selectively in the prefrontal and temporal cortical regions
which, in rhesus monkeys, have especially high concentra-
tions of dopamine receptors among cortical areas studied
(31). In humans, these areas are also critically involved in
cognitive and affective functions (for review see refs. 32 and
33) and are the areas most often compromised in schizophre-
nia (9, 34, 35). All of these considerations suggest that the D1
receptors in these regions may be one of the major sites to
which neuroleptic treatment is and/or should be directed.

Note Added in Proof. Since this paper was submitted for publication,
an article by Malmberg et al. (36) came to our attention. It shows
that clozapine binds to the short isoform of the D2A-dopaminergic recep-
tor with an affinity comparable to that for D4 receptors. It has also
been shown (37) that while the majority of the striatal D2A receptors
belong to the long type, the short isoform is predominant among D2A
receptors in the cerebral cortex (particularly in those areas where we
detected neuroleptic-induced increase in D2A receptors). Thus, the
clozapine-induced up-regulation of D2A sites, detected in the present
study, may reflect an interaction of this drug with the D4 receptor,
the short isoform of the D2A receptor, or both.

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received from Astra Pharmaceutical (Sodertalje, Sweden).

481–483.
3. Runnion, N. M. J., Hall, M. D., Mann, S., Flemingier, S.,
Pharmacol. 34, 2755–2763.
4. O’Dell, S. J., Hoste, G. J., Widmark, C. B., Shapiro, R. M.,
82, 93–109.
6. Memo, M., Pizzi, M., Missale, C., Carruba, M. O. & Sspano,
359–363.
8. Laruelle, M., Jaskiw, G. E., Lipska, B. K., Kolachana, J. E.
10. Davis, K. L., Kahn, R. S., Grant, K. O. & Davidson, M.
12. Pflug, B., Bartels, M., Baner, H., Bunse, J., Gallhofer, B.,
Haus, S., Kanzow, W. T., Lkieser, E., Kufferle, B., Stein,
Scand. 82 (Suppl. 358), 142–146.
Economics, Montville, NJ), 47th Ed.
Drug Therapy (Little-Brown, Boston).
17. Lidow, M. S., Goldman-Rakic, P. S., Gallager, D. W. &
261, 1282–1290.
53–67.
21. VanTol, H. H. M., Bunzow, J. R., Guan, H. C., Sunahara,
22. Meador-Woodruff, J. H., Mansour, A., Healy, D. J., Kuehn,
R., Zhou, Q. Y., Bunzow, J. R., Akil, H., Civelli, O. &
184–189.
24. Baczopoulos, N. C., Spokes, E. G., Bird, E. & Roth, R. H.
26. Gerfen, C. R., Engler, T. M., Mahan, L. C., Susel, Z., Chase,
1429–1431.
28. Seeman, P., Niznik, H. B., Guan, H. C., Booth, G. & Ulipian,
(Suppl. 4), 14–16.
31. Lidow, M. S., Goldman-Rakic, P. S., Rakic, P. & Innis, R. B.
Plum, F. (Am. Physiol. Soc., Bethesda, MD) Section 1, Vol.
5, pp. 373–417.
35. Benton, M. E., Kikins, R., Jolesz, F. A., Pollock, S. D.,
LeMay, M., Wible, C. G., Hokama, H., Martin, J., Metcalf,
327, 604–612.
37. O’Malley, K. L., Mack, K. J., Gangelman, K. Y. & Tood,