Induction of c-fos mRNA in Rat Medial Prefrontal Cortex by Antipsychotic Drugs: Role of Dopamine D2 and D3 Receptors

Kalpana M. Merchant, Lana M. Figur and Dawna L. Evans
CNS Diseases Research, Upjohn Laboratories, 301 Henrietta Street, Kalamazoo, MI 49001, USA

The present studies compared the effects of acute and chronic administration of haloperidol or clozapine on c-fos mRNA expression in the rat medial prefrontal cortex. Acute administration of clozapine, but not haloperidol, robustly increased c-fos mRNA expression in the infralimbic and prelimbic cortex of the rat. Even though most c-fos mRNA-expressing neurons in the clozapine-treated animals were localized in deep cortical layers, labeled neurons were found organized into several cell bridges connecting the superficial and deep layers of the cortex. After chronic treatment with clozapine, c-fos mRNA was reduced by ~60% of that seen acutely; however, the columns of c-fos mRNA expressing neurons did not show the same magnitude of tolerance. Haloperidol had no significant effect even after chronic treatment. We examined further the role of dopamine D2 versus D3 receptors in c-fos gene induction in the infralimbic cortex by studying the acute effects of remoxipride and U-99194A. Remoxipride, a selective D2 antagonist, induced c-fos mRNA at very low doses and lost its ability to alter c-fos mRNA levels at higher doses. Interestingly, U-99194A, an antagonist with 20-fold selectivity for D3 over D2 receptors, also produced greater induction of c-fos mRNA at lower doses. We hypothesize that blockade of D3 receptors may enhance c-fos gene expression in the medial prefrontal cortex but that of D2 receptors may prevent the same.

Introduction
Antipsychotic drugs (APDs) used in the treatment of schizophrenia are broadly classified into two categories—typical and atypical—based on their clinical effects (reviewed by Deutch et al., 1991; Gerlach, 1991; Kane and Freeman, 1994). The atypical APDs, typified by clozapine, differ from the classical neuroleptics (e.g., haloperidol) due to their ability (i) to relieve psychosis in neuroleptic-resistant patients, (ii) to display better efficacy at treating negative symptoms, and (iii) to produce little or no EPS. It has been proposed that the distinct clinical profile of typical versus atypical APDs may result from their ability to target preferentially distinct neuroanatomic sites and/or dopamine receptor subtypes. In support of differential anatomical targets, these two classes of drugs produce distinct patterns of induction of the immediate early gene, c-fos, in the rat forebrain regions. Thus clozapine and other new putative atypical APDs, but not neuroleptics, increase Fos-like immunoreactivity (Fos-IR) in the medial prefrontal cortex (Robertson and Fibiger, 1992; Robertson et al., 1994; Fink-Jensen and Kristensen, 1994; Deutch and Duman, 1995). On the other hand, the dorsolateral neostriatum (DLSt) shows an induction of c-fos mRNA or Fos-IR by APDs with greater EPS than clozapine (Miller, 1990; Deutch et al., 1992; Merchant and Dorsa, 1992a, b, 1993; Nguyen et al., 1992; Robertson and Fibiger, 1992; Fink-Jensen, 1994; MacGibbon, 1994; Robertson et al., 1994; Sebens et al., 1995). Finally, in the nucleus accumbens, all clinically efficacious APDs increase Fos-IR (Deutch et al., 1992; Robertson and Fibiger, 1992; Fink-Jensen and Kristensen, 1994; MacGibbon, 1994; Robertson et al., 1994; Sebens et al., 1995). Interestingly, the gene encoding the neuroactive peptides, neurotensin/neuromedin N (NT/N), appears to be at least one of the transcription targets of Fos-like proteins in the striatum (Merchant, 1994; Merchant and Miller, 1994; Robertson et al., 1995). Hence, induction of NT/N mRNA in the DLSt and the nucleus accumbens also shows the same pattern of region-specific responses to typical versus atypical APDs as that of Fos-IR (Merchant et al., 1992a,b; Merchant and Dorsa, 1993).

In the present study, we have characterized further the medial prefrontal cortical effects of clozapine and haloperidol by studying alterations in c-fos mRNA expression in distinct cytoarchitectonic regions of the medial prefrontal cortex. Additionally, the acute and chronic effects of haloperidol and clozapine on c-fos mRNA levels in the medial prefrontal cortex were compared since previous studies have shown a region-specific tolerance in the induction of Fos-IR after chronic treatment (Sebens et al., 1995).

The pharmacological mechanisms underlying the differential effects of typical and atypical APDs on c-fos gene induction in the medial prefrontal cortex remain unclear (Deutch and Duman, 1995). Among the dopamine D2-like receptors (i.e., D2, D3, and D4), blockade of D3 and D4 is hypothesized to impart the atypical biochemical and clinical profiles to APDs (Seeman, 1992; Schwartz et al., 1993). Recently Deutch and Duman (1995) have reported that acute treatment with non-selective blockers of D2-like receptors does not produce Fos induction in the medial prefrontal cortex. However, the role of subtypes of D2-like receptors in the induction of Fos in the medial prefrontal cortex has not been reported.

In the present study, we used currently available non-proprietary pharmacological tools to investigate the potential roles of dopamine D2 and D3 receptors in induction of c-fos mRNA in the medial prefrontal cortex by APDs. First, the acute effects of remoxipride on prefrontal cortical c-fos mRNA expression were examined since among the dopamine D2-like receptors, this drug shows moderate but selective affinity for only D2 receptors (Hall et al., 1986). Additionally, remoxipride is an APD with an atypical biochemical and clinical profile (Ahlfors et al., 1990; Lewander et al., 1990; Mendlewicz et al., 1990; Ogren et al., 1990; Walinder and Holm, 1990). Next we studied the effects of U-99194A, a 2-aminoindan with a 20-fold selectivity for D3 over D2 receptors (Waters et al., 1993). Although relatively low, this degree of in vitro selectivity appears to impart a distinct profile of in vivo biochemical and behavioral effects to U-99194A as compared to non-selective D2-blockers. Thus, unlike haloperidol, U-99194A does not produce catalepsy even at very high doses and is a behavior stimulant at doses used in the present study (Waters et al., 1993, 1994). Similar behavioral stimulation is seen with low doses of nafadotride, a compound with a 10-fold preference for D3 over D2 receptors.
and is thought to be due to blockade of postsynaptic D3 receptors (Griffon et al., 1995). These data suggest that it may be possible to achieve in vivo selectivity for D3 receptors by using a dose range of U-99194A that produces behavioral stimulation rather than catalepsy or behavioral inhibition as seen with non-selective D2 antagonists.

Materials and Methods

Animals and Treatment
Male Sprague-Dawley rats (Charles River) were used for all studies described here. Rats were maintained in a controlled environment with 12 h light/dark cycle with free access to laboratory chow and tap water. To compare the acute and chronic effects of APDs, animals were implanted with s.c. osmotic minipumps (Alzet 2ML4) releasing vehicle (60 μl/day), haloperidol (1 mg [2.7 μmol/kg/day]) or clozapine (20 mg [61 μmol/kg/day]). The vehicle in all cases was sterile water adjusted to pH 5.2 with lactic acid. On day 28, six animals each from the vehicle, haloperidol, and clozapine group were killed by decapitation. The remaining chronic vehicle-treated animals were treated acutely with the vehicle (1 ml/kg), haloperidol (1 mg/kg, i.p.), or clozapine (20 mg/kg, i.p.), and were killed 1 h later.

For the acute studies examining the effects of remoxipride and U-99194A, separate groups of rats (n = 5 or 6) were treated with remoxipride (0.3, 0.6, or 2.5 mg/kg, i.e., 0.9, 1.8, or 7.5 μmol/kg, i.p.), U-99194A (8, 16, or 32 mg/kg, i.e., 25, 50, or 100 μmol/kg, i.p.) or the pH-adjusted vehicle (1 ml/kg, i.p.). For the U-99194A study, one group of rats was injected with clozapine (20 mg/kg, i.p.) for comparison. Rats were killed by decapitation 1 h after the treatment. Brains were rapidly removed, frozen on dry-ice and stored at -80°C until assayed for c-fos or NT/N mRNA levels by in situ hybridization histochemistry.

In Situ Hybridization Histochemistry and Autoradiography
Brains were cut into 20 μm thick slices on a cryostat. Detection of c-fos mRNA and NT/N mRNA was accomplished using 35S-labeled oligonucleotide or antisense RNA probes, respectively, as described previously (Merchant et al., 1992a; Merchant and Dorsa, 1993). Briefly, under RNase-free conditions, sections were fixed in 4% paraformaldehyde, acetylated with acetic anhydride in triethanolamine buffer, dehydrated in a graded alcohol series, delipidated in chloroform, and rehydrated in 95% ethanol. The c-fos oligonucleotide probe was end-labeled using terminal deoxynucleotidyl transferase and 35S-labeled dATP to a specific activity of 7.5-15 x 106 d.p.m./μmol probe. The NT/N cRNA probe was transcribed in vitro and labeled with 35SUTP to a specific activity of 40-80 x 106 d.p.m./μmol probe. Hybridization was carried out at 37 and 52°C, respectively, for c-fos and NT/N mRNA at a concentration of 2 pmol probe/ml of hybridization buffer. After an overnight incubation, high-stringency washes were performed in 1x SSC at 65°C for c-fos and 0.1x SSC at 55°C for NT/N mRNA. Slides were then dehydrated through a graded alcohol series, air-dried and film autoradiograms were generated by apposing the sections to Hyperfilm βmax (Amersham). Sections were then coated with Kodak NTB-2 nuclear track emulsion, developed in D-19 (Kodak), fixed, and counterstained with cresyl violet.

Analysis of Hybridization Signal
Schematic diagrams of the rat brain coronal sections depicting the regions of the prefrontal cortex and the neostriatum analyzed are shown in Figure 1. Emulsion-coated sections were used to analyze c-fos mRNA expression in the medial prefrontal cortex. The number of labeled cells in a specified division of the prefrontal cortex as well as the number of grains associated with the labeled cells were determined using a computer-assisted image analysis system (MCID or Image 1). For counting the number of labeled cells, dark-field images at 20x magnification were used. For grain density over neurons, dark-field images at 40x magnification were used. The average grain density per labeled cell in each region was determined by comparing the corresponding dark-field image.

Data Presentation and Statistical Analysis
Quantitative analysis was carried out using three sections/animal. Data from the three sections were pooled to obtain the average hybridization signal/animal. For each treatment group, the average hybridization signal was computed and statistical analysis performed using one-way analysis of variance (ANOVA). Following a significant difference in the variance (P < 0.05), Scheffe's or Fisher's least-square difference tests were applied to identify groups significantly differing from each other at P < 0.05.

Results

Comparison of Acute and Chronic Effects of Haloperidol and Clozapine
A significant induction of c-fos mRNA in the medial prefrontal cortex of the rat was observed after acute administration of clozapine but not haloperidol (Figs 2 and 3). Within the medial prefrontal cortical regions, maximal increases were observed in the infralimbic and ventral prelimbic regions. The dorsal anterior cingulate cortex (dACg) did not show consistent induction in all animals. As described previously for Fos immunoreactivity (Deutch and Duman, 1995), most c-fos mRNA-expressing neurons were located in deep cortical layers and had the appearance of large pyramidal cells. Layers II and III also showed induction of c-fos mRNA expression, albeit less than that seen in the deep layers. Microscopic analysis of the tissue sections revealed the presence of two or three cell bridges formed by c-fos mRNA-expressing neurons connecting the deep and superficial cortical layers (Fig. 4). One of these columns was found within the boundaries of the infralimbic cortex and one or two others were usually observed within the prelimbic cortex. After the chronic clozapine treatment, there was a significant reduction (~60%) in the number of cells expressing c-fos mRNA in the infralimbic cortex (Fig. 3). The density of grains associated with these cells was also significantly reduced. However, the cell bridges formed by the c-fos mRNA-expressing neurons did not...
Figure 2. Expression of c-fos mRNA in the medial prefrontal cortex after acute and chronic haloperidol and clozapine. Representative autoradiograms demonstrate the expression of c-fos mRNA in the medial prefrontal cortex in animals treated for 1 h with an acute i.p. dose of vehicle (a), haloperidol (1 mg/kg) (b), or clozapine (20 mg/kg) (c), or after 28 days of treatment via s.c. implanted osmotic minipumps containing vehicle (A), haloperidol (1 mg/kg/day) (B), or clozapine (20 mg/kg/day) (C). Arrowheads indicate infralimbic (IL) and prelimbic (PL) cortex.

show the same degree of tolerance (reduced by ~35% of the acute response) as judged by the average autoradiographic grain density of neurons within the columns (Fig. 5).

Effects of Remoxipride on c-fos mRNA Expression in the Medial Prefrontal Cortex
A significant induction in c-fos mRNA expression in the infralimbic cortex was observed only after the low dose (0.3 mg/kg) of remoxipride and not at 0.6 or 2.5 mg/kg (Figs 6 and 7). The regional distribution of remoxipride effects on c-fos mRNA levels was similar to that observed after clozapine, i.e., infralimbic > prelimbic, with little effect in the dorsal anterior cingulate cortex. However, the laminar distribution differed somewhat. Remoxipride-induced c-fos mRNA expression was not as restricted to the deep cortical layers as that observed after clozapine. Additionally, the cell bridges, though visible in most animals, were not as well defined as those after clozapine.

Effects of U-99194A on c-fos mRNA Expression in the Medial Prefrontal Cortex
A single dose of U-99194A produced a robust increase in c-fos mRNA expression in the medial prefrontal cortex at all doses tested (Figs 8 and 9). However, several distinctions from the effects of clozapine were notable: (i) the greatest effect was localized in the dorsal anterior cingulate cortex followed by the infralimbic and ventral prelimbic regions (Fig. 9); (ii) like remoxipride, the effects of U-99194A were seen evenly in both deep and superficial layers; and (iii) the cell bridges, though not as distinctly visible as those in clozapine-treated animals, were apparent in all animals (Fig. 10). The dose–response curve of U-99194A effects displayed interesting regional characteristics with respect to the number of labeled cells. In the infralimbic cortex, an inverted U-shaped curve was observed. Thus, the maximal effect was produced by the medium dose of 16 mg/kg of U-99194A. The highest dose of 32 mg/kg produced an effect that was significantly lower than that produced by 16 mg/kg (Fig. 9). However, the density of autoradiographic grains associated with the cells did not show this dose dependency. Interestingly, in the dorsal anterior cingulate region, all doses appeared to be equally efficacious (Fig. 9).

Effects of Remoxipride and U-99194A on c-fos and NT/N mRNA Expression in the Caudate-Putamen and Nucleus Accumbal Shell
Remoxipride did not alter NT/N or c-fos mRNA expression in the DLSt at any dose tested. However, in the shell sector of the
Acute versus Chronic Effects of Clozapine and Haloperidol in the Medial Prefrontal Cortex

Gene expression was maintained in animals treated chronically with continuous administration of these APDs. Compared to the effect of an acute dose of clozapine, the chronic treatment produced a significantly attenuated response in all medial prefrontal cortical regions. A confounding factor in the apparent tolerance to chronic clozapine administration may be the plasma clozapine concentration generated by an acute i.P. injection versus continuous release from s.c. osmotic pumps. However, the important finding is the relatively persistent level of c-fos mRNA in cell groups organized into columns that appeared to connect the deep and superficial cortical layers after chronic clozapine treatment. The differential tolerance in c-fos gene induction in neurons forming the bridges versus those surrounding the bridge-neurons indicates a pharmacodynamic mechanism underlying the attenuated response to chronic clozapine treatment. Such a columnar distribution of Fos-IR neurons after an acute clozapine treatment has been described recently by Deutch and Duman (1995).

It is possible that the sustained expression of the c-fos gene in the neurons in the infralimbic and prelimbic cortices is related to the persistent biochemical effects of chronic clozapine in the medial prefrontal cortex (Chen et al., 1992; Youngren et al., 1994). Region-specific maintenance of Fos-IR after chronic treatment with clozapine has been reported recently also in subcortical regions (nucleus accumbens and lateral septum) (Sebens et al., 1995). The rat infralimbic and prelimbic cortices appear to be an integral part of the limbic circuit involving the medial prefrontal cortex, the shell sector of the nucleus accumbens, the ventral pallidum, and the mediodorsal thalamus (Sesack et al., 1992). Abnormalities in neural activity in this circuitry have been observed in schizophrenic subjects and hypothesized to contribute to the pathophysiology of schizophrenia (Weinberger et al., 1986; Andreasen et al., 1994). Hence, a sustained increase in c-fos gene expression in the infralimbic/ventral prelimbic cortex and subcortical regions by clozapine indicates that these neurons may contribute to the therapeutic effects of clozapine.

**Effects of D2 Receptor Blockade by Remoxipride on c-fos mRNA Induction in the Medial Prefrontal Cortex**

Like clozapine, acute administration of remoxipride significantly increased c-fos mRNA expression in the infralimbic cortex, albeit only at low doses. Remoxipride, an atypical APD, also shares the property of clozapine of being a weak antagonist at the D2 receptors (Kt = 125 nM) (Hall et al., 1986; Kohler et al., 1990). In fact, the low D2 affinity contributes to significantly lower in vivo D2 occupancy in both rats and human (Farde and von Bahre, 1990; Kohler et al., 1990; Waters et al., 1994) and is thought to underlie its atypical APD biochemical and clinical profile (Ahlfors et al., 1990; Lewander et al., 1990; Mendlewicz et al., 1990; Ogren et al., 1990; Walinder and Holm, 1990). These data and the inverted dose-response curve of remoxipride-induced c-fos mRNA induction seen in the present study have led us to hypothesize that the degree to which dopamine D2 receptors are blocked may be inversely related to the induction of the c-fos gene in the infralimbic cortex. This possibility is supported by the inability of haloperidol, and other high-affinity D2 blockers, to induce c-fos gene expression in the infralimbic cortex (present study, Robertson et al., 1994; Deutch and Duman, 1995). Additionally, in rats remoxipride generates active phenolic metabolites (FLS 797 and FLA 908) that block D2 receptors with significantly higher affinity (Kt = 1.04 and 3.35 nM, respectively) (Mohell et al., 1993; Ogren et al., 1993).

**Discussion**

**Acute versus Chronic Effects of Clozapine and Haloperidol in the Medial Prefrontal Cortex**

As reported previously for Fos-IR (Robertson and Fibiger 1992; Deutch and Duman, 1995), acute administration of clozapine but not haloperidol significantly increased c-fos mRNA levels in the infralimbic and prelimbic cortex. This selective effect of clozapine of c-fos gene expression was maintained in animals...
Figure 4. Presence of cellular bridges of neurons expressing c-fos mRNA after clozapine administration. (A–C) Low magnification (5×) dark-field photomicrograph montages from animals treated with acute vehicle (A), acute clozapine (B) and chronic clozapine (C). For orientation, brain midline is shown by arrows and the corpus callosum is depicted by cc. The photomicrographs show cell bridges (demarcated by arrowheads) formed by c-fos mRNA-expressing neurons after acute and chronic clozapine treatment. Note that the bridges connect the deep and superficial cortical layers. (a–c) The corresponding high magnification (40×) photomicrographs showing the autoradiographic grain density over neurons forming the cell bridges.

Whether the D2-blocking effects of the metabolites contribute to the pharmacological profile of reloxipride remains to be seen.

Recently, Robertson et al. (1994) used the immunohistochemical technique to show an increase in the number of Fos-IR neurons in the medial prefrontal cortex after 3 mg/kg, but not 1.5 mg/kg, s.c., of remoxipride. The apparent discrepancy in the dose–response relationship between the results of the present study and that of Robertson et al. (1994) may be due to the difference in the routes of administration or to the participation of other Fos-like antigens.

Unlike remoxipride itself, its phenolic metabolites also display a high affinity for the D3 receptors (Ki = 969, 0.38, and 7.39 for remoxipride, FLA 797, and FLA 908, respectively) (Malmberg et al., 1993; Mohell et al., 1993). These phenolic metabolites also show greater distribution in the brain as compared to the plasma (Ogren et al., 1993). Finally, i.p. administration of reloxipride generates greater amounts of these metabolites as compared to the s.c. route (Ogren et al., 1993). Whether the levels of phenolic metabolites in the brain are sufficient to block D3 receptors and thereby participate in the induction of the c-fos gene in the rat medial prefrontal cortex remains to be tested. Similarly, other pharmacological properties of reloxipride, such as blockade of sigma receptors (Hall et al., 1986) or its intrinsic efficacy at the D2 receptors (Ahlenius et al., 1993), may contribute also to the induction of c-fos expression in the medial prefrontal cortex.

Figure 5. Analysis of autoradiographic grain density in the cell bridges showing c-fos mRNA expression after acute and chronic clozapine. In order to determine the persistence of c-fos mRNA expression in neurons arranged in a columnar fashion in the infralimbic/prelimbic cortex (see text for details), the average density of autoradiographic grains associated with these cells was determined as an index of the level of c-fos mRNA per neuron. **P < 0.01 as compared to the corresponding vehicle-treated groups. CLZ, clozapine.
Role of D3 Receptors on c-fos mRNA Induction in the Medial Prefrontal Cortex

Opposing roles of D3 and D2 receptors in regulation of c-fos mRNA expression in the medial prefrontal cortex were also suggested by the inverted dose-response curve seen in the effects of the D3-prefering antagonist, U-99194A. These results are consistent with the hypothesis presented above that the degree of blockade of D2 receptors is negatively related to c-fos gene expression in the infralimbic cortex of the rat. That the highest dose of U-99194A used here can produce effects mediated via the blockade of D2 receptors was evident in the induction of NT/N mRNA in the DLSt and nucleus accumbens in some animals treated with this dose. This is based on previous studies that have shown that APDs with high affinity for D2 receptors induce NT/N gene expression in both the accumbal shell and the DLSt (Merchant et al., 1992a). Additionally, antisense oligonucleotide studies indicate that the DLSt response is mediated by D2 receptors (K. M. Merchant, unpublished observations). If the degree of the blockade of D2 receptors determines the anatomical pattern of c-fos and NT/N gene expression in the prefrontal cortex and the subcortical structures, respectively, high doses of clozapine that induce NT/N mRNA in the DLSt (Merchant et al., 1992a) should produce little or no effect in the medial prefrontal cortex. This possibility is being examined.

It is important to note that U-99194A did not induce NT/N mRNA expression in the accumbal shell at doses that produced enhanced levels of c-fos mRNA in the medial prefrontal cortex. These data contradict a recent report (Diaz et al., 1994) that suggested that D3 receptor mRNA and NT/N mRNA-expressing neurons in the ventral accumbal shell are co-localized, and that expression of the NT/N gene in this region may be decreased by drugs that block D3 receptors. In fact, subregional analysis of NT/N gene expression within the accumbal shell showed no evidence of reduction in NT/N mRNA levels by haloperidol, remoxipride, or clozapine (data not shown). One possible explanation for the differences between our results and those of Diaz et al. (1994) may be the dose of haloperidol employed by the latter investigators (20 mg/kg, i.p.).

Finally, some of the subregional patterns of distribution of c-fos mRNA-expressing cells in the medial prefrontal cortex may also provide a functional index of D2 and D3 receptor blockade. For example, clozapine-induced c-fos mRNA expression predominates in deep cortical layers (the exception of the cell bridges). On the other hand, both remoxipride and U-99194A increased c-fos mRNA levels in cortical layers II-VI. Additionally, U-99194A caused the most robust induction in the dorsal anterior cingulate cortex that was not observed after remoxipride or clozapine. Furthermore, all doses of U-99194A produced similar levels of increases in c-fos gene expression in the dorsal anterior cingulate cortex. These data suggest that in this region of the cingulate cortex, expression of c-fos mRNA may not be negatively regulated by D2 receptors.

The medial prefrontal cortical neurons show a very low level of expression of D3 mRNA (Meador-Woodruff et al., 1994). Hence, a robust induction in c-fos gene expression by U-99194A indicates effects mediated via a distant site. Expression of D3 receptors on the ventral tegmental area dopamine neurons that project to the medial prefrontal cortex has been suggested by Sokoloff et al. (1992) but remains controversial (Landwehrmeyer et al., 1993; Meador-Woodruff et al., 1994). One likely possibility may be the paraventricular thalamic nucleus since neurons in this region display selective expression of D3 mRNA (Meador-Woodruff et al., 1994). Additionally, this thalamic region has reciprocal connections with the medial prefrontal cortex and shows a robust induction of Fos protein after an acute treatment with clozapine (Deutch et al., 1995).

In summary, the results presented here show that chronic treatment with clozapine may have a sustained effect in altering the activity of some neurons within the infralimbic cortex of the rat. Although the pharmacological mechanism underlying the induction of c-fos by clozapine was not tested directly, the blockade of D3 receptors appears to lead to an increase in c-fos gene expression in the medial prefrontal cortex. On the other hand, we hypothesize that the blockade of D2 receptors may be negatively linked to c-fos induction in the medial prefrontal

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Figure 6. Remoxipride-induced expression of c-fos mRNA in the medial prefrontal cortex. Representative autoradiograms demonstrate the expression of c-fos mRNA in the medial prefrontal cortex in animals treated for 1 h with an acute i.p. dose of vehicle (A), or remoxipride at 0.3 mg/kg (B), 0.6 mg/kg (C), or 2.5 mg/kg (D). Note the robust induction of c-fos mRNA in the infralimbic/prelimbic cortex after 0.3 mg/kg of remoxipride and the loss of this hybridization signal at higher doses. On the other hand, the dorsal anterior cingulate cortex did not show a significant induction in c-fos mRNA expression at any dose tested.
Figure 7. Quantification of remoxipride-induced c-fos mRNA levels in the infralimbic/ventral prelimbic (IL/VPL) and dorsal anterior cingulate (daCg) cortex. The hybridization signal represented in Figure 8 was analyzed by determining the number of labeled cells and the autoradiographic grain density per cell. *P < 0.02 versus the vehicle-treated group. **P < 0.05, ***P < 0.01 as compared to the effect produced in the group treated with 0.3 mg/kg remoxipride (Remox).

Figure 8. Expression of c-fos mRNA in the medial prefrontal cortex after an acute dose of the D3-prefering antagonist, U-99194A, or clozapine. Rats were treated with a single i.p. dose of vehicle (A), 20 mg/kg of clozapine (B), or U-99194A at 8 mg/kg (C), 16 mg/kg (D), or 32 mg/kg (E), and were killed 1 h after the treatment. Note the robust induction of c-fos mRNA levels in the infralimbic, prelimbic and dorsal anterior cingulate cortex after all doses of U-99194A.

Figure 9. Quantification of U-99194A-induced c-fos mRNA levels in the infralimbic/ventral prelimbic (IL/VPL) and dorsal anterior cingulate (daCg) cortex. The hybridization signal represented in Figure 9 was analyzed by determining the number of labeled cells and the autoradiographic grain density per cell. Note that the highest dose of U-99194A produced a significantly lower expression of c-fos mRNA in the IL/VPL cortex than that produced by 16 mg/kg of the drug. *P < 0.05, **P < 0.001 versus the corresponding vehicle-treated group. **P < 0.05 as compared to the effect produced after 16 mg/kg of U-99194A.
Figure 10. Presence of c-fos mRNA expressing cell bridges after acute administration of U-99194A. Rats were treated as described in Figure 9. Panels on the left are montages of low magnification (6×), dark-field photomicrographs from animals treated with acute vehicle (A), acute clozapine (B), or U-99194A at 8 mg/kg (C), 16 mg/kg (D), or 32 mg/kg (E). For orientation, brain midline is shown by arrows and the corpus callosum is depicted by cc. Note that the cell bridges (demarcated by arrowheads) were more distinct after clozapine treatment as compared to the effects of U-99194A.
cortex. It is emphasized, however, that future studies using other chemically diverse, selective D2 and D3 agonists and antagonists as well as approaches such as antisense knockdown are necessary (i) to confirm the present hypotheses and (ii) to characterize better the role of D2 and D3 receptors in APD effects.

Notes
Address correspondence to Dr Kalpana M. Merchant, CNS Diseases Research, Upjohn Laboratories 7251-209-506, 301 Henrietta Street, Kalamazoo, MI 49008, USA.

References


Figure 11. Expression of NT/N mRNA expression in the nucleus accumbens-shell and the DLSt after acute remoxipride and U-99194A. Animals were treated as described in Figures 6 and 8 and expression of NT/N mRNA was quantified by optical density measurement of film autoradiograms; the regions analyzed are shown schematically in Figure 1. *P < 0.05, **P < 0.001 versus the corresponding vehicle-treated group.
Lewander T, Westebergh S-E, Morrison D (1990) Clinical profile of
remoxipride—a combined analysis of a comparative double-blind
multicentre trial programme. Acta Psychiatr Scand 82 (Suppl

MacGibbon GA, Lawlor PA, Bravo, R, Dragunow M (1994) Clozapine and
haloperidol produce a differential pattern of immediate early gene
expression in rat caudate-putamen, nucleus accumbens, lateral

characteristics of antipsychotic agents interacting with human D2,
D3 and D5 receptors. Mol Pharmacol 45:749–754.

Meador-Woodruff JH, Mansour A, Saul J, Watson SJ (1994) Neuro-
anatomical distribution of dopamine receptor messenger RNAs. In:
Dopamine receptors and transporters: pharmacology, structure, and


Mendelwicz J, de Bleeker E, Cosyns P, Delieu G, Lostra F, Masson A,
Marcens C, Parent M, Peusken J, Suy E, de Wilde J, Wilmotte J, Norgard
J (1990) A double-blind comparative study of remoxipride and
haloperidol in schizophrenic and schizophreniform disorders. Acta

Merchant KM (1994) c-fos antisense oligonucleotide specifically
attenuates haloperidol-induced increases in neurotensin/neuromedin
N mRNA expression in rat dorsal striatum. Mol Cell Neurosci
5:336–344.

c-fos gene expression by typical vs. atypical antipsychotics. Proc Natl
Acad Sci USA 90:3447–3451.

mRNAs in rat neostriatal neurons following acute haloperidol. Mol

Merchant KM, Dobie DJ, Dorsa DM (1992a) Expression of the
proneurotensin gene in the rat brain and its regulation by

Merchant KM, Dobner PR, Dorsa DM (1992b) Differential effects of
haloperidol and clozapine on neurotensin gene transcription in rat

Merchant KM, Dobie DJ, Filloux F, Totzke M, Aravagiri M, Dorsa DM
(1994) Effects of chronic haloperidol and clozapine treatment on
neurotensin and c-fos mRNA in rat neostriatal subregions. J Pharmacol
Exp Ther 271:460–471.

Miller JC (1990) Induction of c-fos mRNA expression in rat striatum by

Binding characteristics of remoxipride and its metabolites to

Differential expression of c-fos and Zif 268 in rat striatum following
haloperidol, clozapine and amphetamine. Proc Natl Acad Sci USA
89:4270–4274.

Neuropsychopharmacological and behavioral properties of remoxipride

blocking activity and plasma concentrations of remoxipride and its


Robertson GS, Fibiger HC (1992) Neuroleptics increase c-fos expression
in the forebrain: contrasting effects of haloperidol and clozapine.

Robertson GS, Matsumura H, Fibiger HC (1994) Induction of Fos-like
immunoreactivity in the forebrain as predictors of atypical

Robertson GS, Tetzlaff W, Bedard A, St-Jean M, Wigle N (1995) c-fos
mediates antipsychotic-induced gene expression in the rodent

receptor: basis and clinical aspects. Clin Neuropharmacol 16:
295–314.

induction in rat forebrain regions after acute and long-term

Seeman P (1992) Dopamine receptor sequences: therapeutic levels of
neuroleptics occupy D2 receptors, clozapine occupies D4. Neuro-
psychopharmacology 7: 261–284.

Sesack SR, Deutch AX, Rooth RH, Bunney BS (1992) Topographical
organization of the efferent projections of the medial prefrontal cortex
in the rat: an anterograde tract-tracing study with Phaseolus vulgaris

differential third dopamine receptor (D3) as a novel target for antipsychotics.

Walinder J, Holm AC (1990) Experience of long-term treatment with
remoxipride: efficacy and tolerability. Acta Psychiatr Scand 82 (Suppl

Waters N, Lobberg L, Haadsma-Svensson S, Svensson K, Svensson C,
Carlsson A (1994) Differential effects of dopamine D2 and D3 receptor
antagonists in regard to release, in vivo receptor displacement and

Waters N, Svensson K, Haadsma-Svensson S, Smith MW, Carlsson A

Weinberger DR, Berman KP, Zec RF (1986) Physiological dysfunction of
the dorsolateral prefrontal cortex in schizophrenia. 1. Regional

Youngren KD, Moghaddam B, Bunney BS, Roth RH (1994) Preferential
activation of dopamine overflow by chronic clozapine treatment.