ELSEVIER

Clozapine- and Olanzapine-induced Fos Expression in the Rat Medial Prefrontal Cortex is Mediated by β-Adrenoceptors

Kazuyo Ohashi, M.D., Ph.D., Takashi Hamamura, M.D., Ph.D., Youmei Lee, M.D., Yutaka Fujiwara, M.D., Ph.D., Hiroshi Suzuki, M.D., and Shigetoshi Kuroda, M.D., Ph.D.

The atypical neuroleptics, clozapine and olanzapine, have superior therapeutic efficacy against the negative symptoms of schizophrenia, compared with the typical neuroleptics. Recently, it has been suggested that the ability of clozapine and olanzapine to induce Fos expression in the medial prefrontal cortex (mPFC), contribute to their therapeutic efficacy. However, the mechanisms underlying the neuropharmacological effects of clozapine and olanzapine in the mPFC remain elusive. In the present study, we demonstrate that clozapine- and olanzapine-induced Fos expression in the mPFC are inhibited by propranolol. We also show that clozapine and olanzapine induce Fos expression in the locus coeruleus. These results suggest that clozapine and olanzapine increase noradrenaline release by stimulating noradrenergic neuronal activity in the locus coeruleus and, consequently, increased noradrenaline induce Fos expression in the mPFC via β-adrenergic receptors. This postulated sequence may be one of mechanisms by which clozapine-like atypical neuroleptics are more effective for the negative symptoms of schizophrenia. ***[Neuropsychopharmacology 23:162–169, 2000]***

KEY WORDS: *Clozapine; Olanzapine; Noradrenaline; β-Adrenergic receptors; Atypical antipsychotics; Immediate-early genes; Fos, Prefrontal cortex; Negative symptom; Schizophrenia*

Clozapine, a prototype atypical antipsychotic compound, is effective not only against the positive, but also against the negative symptoms of schizophrenia (Claghorn et al. 1987; Kane et al. 1988) which are usually resistant to typical neuroleptics. The mechanisms underlying this action, although investigated intensively, remain to be established. Recent studies involving the Fos protein have demonstrated that clozapine induces a much larger increase in the number of neurons that exhibit Fos-like immunoreactivity (LI) in the medial prefrontal cortex (mPFC) than do typical neuroleptics (Deutch and Duman 1996; Fink-Jensen and Kristensen 1994; Fink-Jensen et al. 1995; Guo et al. 1995; Kurokawa et al. 1997; Robertson and Fibiger 1992; Robertson et al. 1994; Wan et al. 1995). This regionally specific clozapine effect sets it apart from typical neuroleptics.

Although simple neuronal depolarization or increase in firing rate per se are not enough to induce c-fos in many neurons, other factors such as phenotype of neuronal cell are also required for c-fos expression, Fos protein is thought to be a marker of activated neurons in specified subset of neurons (Sagar et al. 1988; Dragunow and Faull 1989; Grant et al. 1992; Sharp et al. 1993). Therefore, it can be postulate that clozapine may enhance neuronal activity in the mPFC. In connection with the hypothesis that hypofrontality may be respon-

From the Department of Neuropsychiatry (KO, TH, YL, HS, SK), Okayama University Medical School, Shikata-cho Okayama, Japan; and Takaoka Hospital (YF), Nishi-imazyuku Himeji, Japan.

Address correspondence to: Dr. T. Hamamura, Department of Neuropsychiatry, Okayama University Medical School, 2–5–1 Shikata-cho, Okayama, 700–8558 Japan.

Received November 9, 1999; revised February 8, 2000; accepted February 17, 2000.

0893-133X/00/$–see front matter
PII S0893-133X(00)00105-6

sible for the negative or deficit symptoms of schizophrenia (Weinberger 1988), the preferential induction of Fos-LI in the mPFC is postulated to be one of the mechanisms by which clozapine reduces the negative symptoms of schizophrenia (Robertson et al. 1994). If this is the case, it is important to determine the neuropharmacological mechanisms by which clozapine induces Fos expression in the mPFC. Although clozapine is known to have an affinity for various neurotransmitter receptors, such as noradrenergic, dopaminergic, histaminergic, muscarinic and 5-hydroxytryptamine (5-HT) receptors, the precise neuropharmacological action of clozapine that results in the induction of Fos expression in the mPFC is unknown. Since clozapine administration increases the firing rate of noradrenergic cells in the locus coeruleus (LC) and noradrenaline release from nerve terminals in the mPFC (Ramirez and Wang 1986; Li et al. 1998; Westerink et al. 1998), it is possible that clozapine-induced noradrenaline release activates cortical neurons in the mPFC.

The recently developed clozapine-like atypical neuroleptic, olanzapine, is also effective in treating the negative symptoms of schizophrenia (Beasley et al. 1996; Hamilton et al. 1998) and shares similar neuropharmacological profiles with clozapine, including receptor binding (Bymaster et al. 1996a), noradrenaline release in the mPFC (Li et al. 1998; Westerink et al. 1998) and preferential Fos induction in the mPFC (Robertson and Fibiger 1996; Sebens et al. 1998). Previous studies have shown that Fos expression induced by noradrenaline in the frontal cortex is mediated mainly by β-adrenoceptors (Bing et al. 1992; Stone and Zhang 1995). Therefore, we hypothesize that clozapine and olanzapine stimulate noradrenaline release and induce Fos expression in the mPFC via β-adrenoceptors. To test this hypothesis, we examined whether the β-adrenoceptor antagonist, propranolol, could block clozapine- or olanzapine-induced Fos expression in the mPFC. We also examined Fos induction in the LC to confirm that these agents activate the noradrenergic neurons in the LC.

METHODS

Drug Treatment

All efforts were made to minimize both animal suffering and the number of animals used, in accordance with the Guidelines for Animal Experiments of Okayama University Medical School. Male Wistar rats (Clea, Japan; body weight: 250–270 g) were used. The animals were housed two per cage with a 12-h light/12-h dark cycle (lights on at 7:00 A.M.), and each rat was adapted to handling every day for five minutes during the week prior to the experiment. There were seven treatment groups (n = 7/group): 1) saline (1 ml/kg, subcutaneously (s.c.)); 2) saline followed by 0.2% acetic acid (1 ml/kg, s.c.); 3) saline followed by clozapine (20 mg/kg, s.c.); 4) saline followed by olanzapine (5 mg/kg, s.c.); 5) propranolol (5 mg/kg, s.c.) followed by 0.2% acetic acid (1 ml/kg, s.c.); 6) propranolol (5 mg/kg, s.c.) followed by clozapine (20 mg/kg, s.c.); and 7) propranolol (5 mg/kg, s.c.) followed by olanzapine (5 mg/kg, s.c.).

Second treatments were administered thirty minutes after the first treatments. Clozapine (Sigma, St. Louis, MO) and olanzapine (generous gift from Eli Lilly Co., Indianapolis, IN) was dissolved in 10 μl of 20% acetic acid and then diluted to 1 ml with distilled water (clozapine; 20 mg/ml, olanzapine 5mg/ml). DL-propranolol HCl (Sigma) was dissolved in saline (5 mg/ml). Two hours after the second injection, all of the animals were anesthetized deeply with pentobarbital. They were then perfused through the ascending aorta with 200 ml of saline followed by 250 ml of 4% paraformaldehyde in 0.1 M phosphate buffer. Their brains were removed immediately after perfusion and then immersed in the same fixative. After a 24-h fixation period, coronal sections (30 μm) were cut from each brain using a Microslicer (DSK, Kyoto, Japan).

Immunohistochemistry

Sections were incubated with primary antiserum (1: 2500 dilution in phosphate-buffered saline at pH 7.4 containing 0.3% Triton X-100, 0.05% sodium azide, and 2% normal rabbit serum) for 72 h at 4°C. The primary polyclonal antibody (OA-11–824; Genosys Biotechnologies, UK) was raised in sheep against residues 2–16 of the N-terminal region of the Fos protein. Immunoreactivity in the sections was visualized using the diaminobenzidine (DAB)-nickel method, described in detail elsewhere (Hamamura et al. 1997). Since the antibody recognizes both Fos and some related antigens, positive staining is referred to as Fos-LI. Sections including the LC were incubated in a 1:5000 dilution of an anti-tyrosine hydroxylase monoclonal antibody (Boehringer Mannheim Biochemica) overnight at room temperature. Tyrosine hydroxylase-LI in these sections also was visualized using a DAB method. All sections were mounted on chrome-alum/gelatin-coated slides. Sections including the prefrontal cortex were left to dry overnight, counterstained with neutral red, and then coverslipped.

Quantification

The numbers of neurons showing Fos-LI were counted in six sequential sections from each of the two areas of the brain according to the antero-posterior coordinates specified by Paxinos and Watson (1986) relative to the bregma and associated structures: +2.7 for cingulate cortex area 3 (Cg3) and - 10.04 for the LC. In the Cg3, Fos-LI was quantified at × 95 magnification by counting the number of neurons showing Fos-LI within a 380 ×

380 μm^2 grid (Figure 1). Fos-LI in the LC was determined by counting the number of nuclei showing Fos-LI within tyrosine hydroxylase-positive neurons. Fos-LI neuronal counts were performed bilaterally on each of the six sections through each region from each animal by an investigator who was unaware of the treatment group. This resulted in a total of 12 determinations of the number of neurons showing Fos-LI within a specified region for each animal. The mean of these 12 determinations was used for subsequent statistical analysis.

Statistical Analysis

A one-way analysis of variance was performed on the number of neurons showing Fos-LI within the Cg3. *Post hoc* individual comparisons were performed using Scheffe's test. Student's t-test was used for analysis of the LC data.

RESULTS

Significant intergroup differences were present for the Cg3 between the saline, saline + vehicle, propranolol + vehicle, saline + clozapine, and propranolol + clozapine groups ($F(4, 30 = 37.74, p < .0001$). A *post hoc* comparison demonstrated that the saline + clozapine group had significantly more neurons showing Fos-LI than the saline + vehicle group, whereas pretreatment with propranolol significantly reduced the number of neurons showing Fos-LI induced by clozapine (Figures 2A, 3A, and 3A′).

Compared with the saline group, the saline + vehicle group showed significantly more Fos-LI-positive neurons. This vehicle-induced Fos expression probably reflects the averse stimulation caused by vehicle injection (0.2% acetic acid). After treatment with propranolol, this increase in Fos expression was largely reversed (Figure 2A). For olanzapine treatment, significant intergroup differences were present for the Cg3 between the saline, saline + vehicle, propranolol + vehicle, saline + olanzapine, and propranolol + olanzapine groups ($F(4,30) = 42.98, p < .0001$). A *post hoc* comparison demonstrated that the saline + olanzapine group showed significantly more Fos-LI-positive neurons than the saline + vehicle group, whereas pretreatment with propranolol significantly reduced the number of neurons showing Fos-LI induced by olanzapine (Figure 2A). The saline + clozapine group had significantly more LC neuronal nuclei showing Fos-LI than the saline group ($t = 11.43, p < .0001$) (Figures 2B, 3B, and 3B′). The saline + olanzapine group had significantly more LC neuronal nuclei showing Fos-LI than the saline group ($t = 13.12, p < .0001$) (Figure 2B).

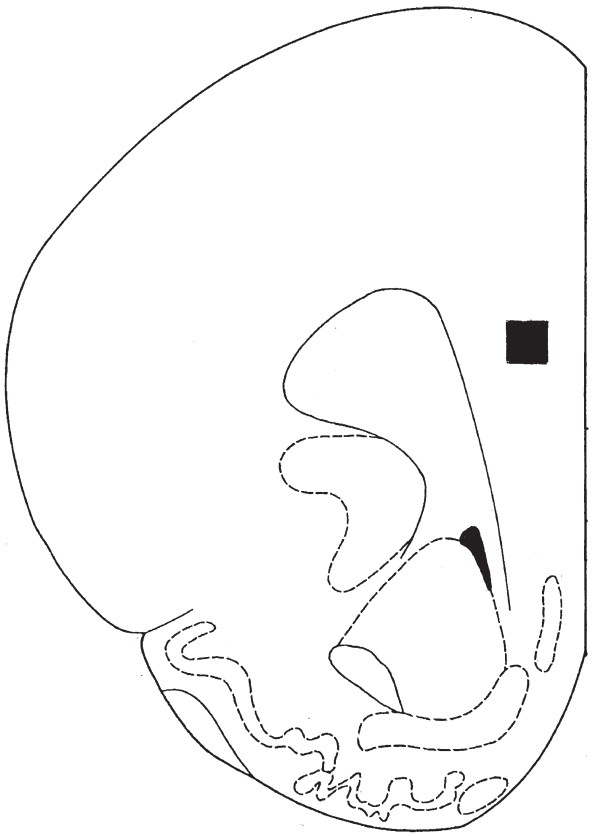

Figure 1. A drawing of section used for the counting of Fos -LI nuclei in the cingulate cortex area 3 (Cg3). A drawing is from Paxinos and Watson (1986).

DISCUSSION

Clozapine has an affinity for various neurotransmitter receptors, including α1- and α2-adrenoceptors (Bymaster et al. 1996a), dopamine D1, D2 (Meltzer et al. 1989), D3 (Sokoloff et al. 1990), and D4 (Van Tol et al. 1991) receptors, as well as 5-HT2A and 5-HT2C receptors; histaminergic receptors (Coward 1992); and muscarinic receptors (Bolden et al. 1991), however, it has a very low affinity for β-adrenoceptors (Bymaster et al. 1996a).

Previous studies have shown that, of these receptors, 5-HT2A/2C receptors, α1-adrenoceptors, muscarinic receptors, and dopamine D2 and D4 receptors are not involved in clozapine-induced Fos expression in the mPFC (Deutch and Duman 1996; Fink-Jensen et al. 1995). So far, β-adrenoceptors have not been investigated. The results of the present study demonstrate that the clozapine-induced Fos expression in the mPFC is mediated by β-adrenoceptors. An early study showed that clozapine increases the levels of 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG) in the rat forebrain (Ader et al. 1980). In addition, Ramirez and Wang (1986) reported that acute clozapine administration to anesthetized rats induced a significant increase

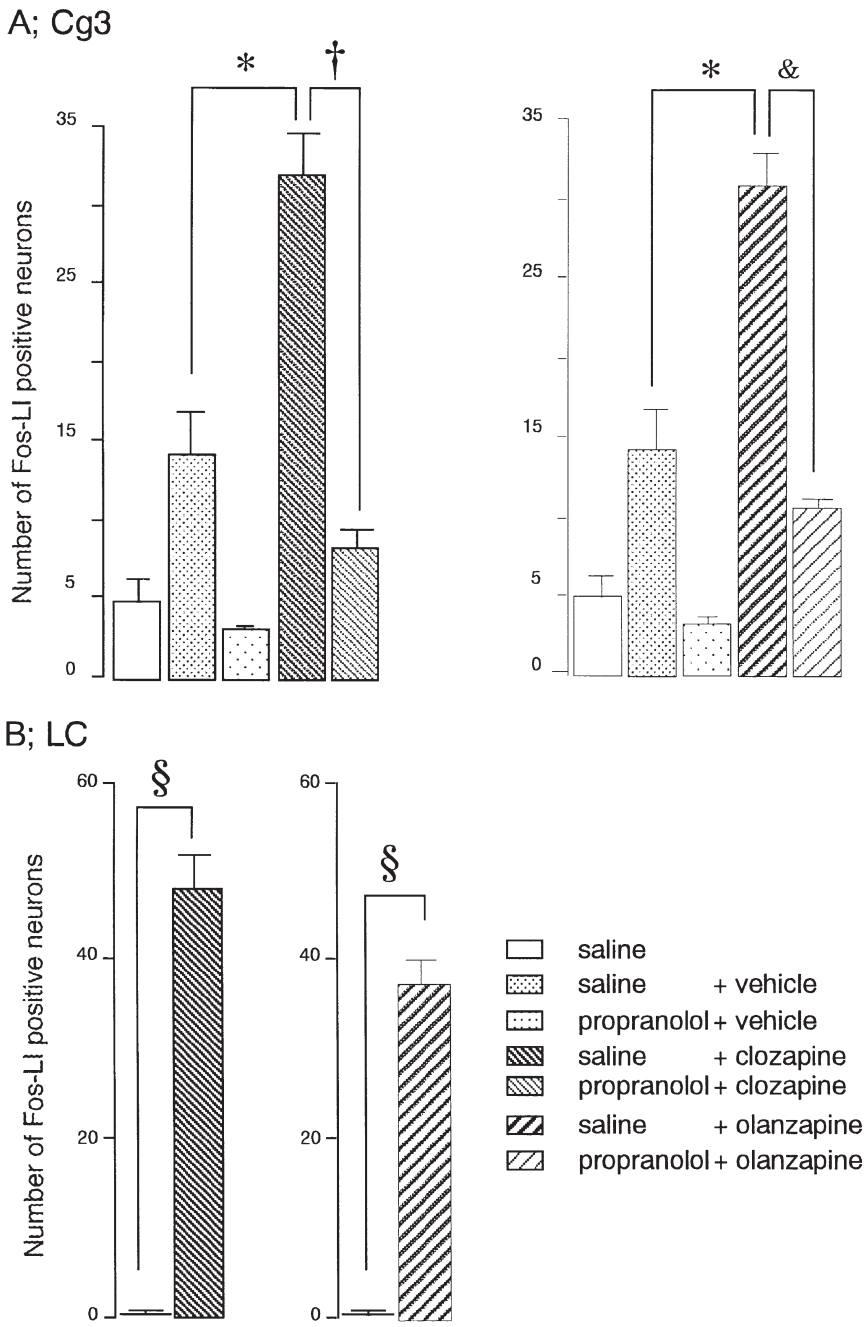

Figure 2. The effect of clozapine on the mean number (± S.E.M.) of nuclei showing Fos-LI in the Cg3 (A) and in noradrenergic neurons in the LC (B). A: Significant differences between groups as determined by one-way analysis of variance followed by Scheffe's *post hoc* test: * $p < .01$, the saline + vehicle vs. the saline + clozapine or saline + olanzapine group; † $p < .01$, the saline + clozapine vs. the propranolol + clozapine group; & $p < .01$, the saline + olanzapine vs. the propranolol + olanzapine group. B: significant differences were also found between the saline and saline + clozapine groups ($t = 11.425$, $p < .0001$) and the saline and saline + olanzapine groups ($t = 13.12$, $p < .0001$). Level of statistical significance, § $p < .01$; Student's t-test.

in both the number and the firing rate of spontaneously active noradrenergic neurons in the LC, from which noradrenergic efferents project into the cerebral cortex (Holes 1990). In agreement with this electrophysiological study in anesthetized rats, the present results demonstrate a significant activation of noradrenergic neurons by clozapine in non-anesthetized rats.

Clozapine shows high affinity for the α2-adrenoceptor (Bymaster et al. 1996a), and α2-adrenoceptors regulate both the firing rate of noradrenergic cells (Cedarbaum and Aghajanian 1977; Freedman and Aghajanian 1984) and the noradrenaline release from nerve terminals (L'Heureux et al. 1986; Van Veldhuizen et al. 1994). Therefore, the α2 antagonistic effect of clozapine at either nerve terminals or neuronal somata in the LC would be expected to increase noradrenaline outflow by blocking negative feedback regulation. Recent dialysis studies have demonstrated that clozapine increases noradrenaline release in the mPFC of freely moving rats (Li et al. 1998; Westerink et al. 1998). Thus, we hypothesize that clozapine may increase the release of noradrenaline and induce Fos expression by stimulating β-adrenoceptors in the mPFC. In contrast, Guo et al. (1995) reported that 6-hydroxydopamine-induced lesions of the dorsal noradrenergic bundle did not affect clozapine-stimulated Fos induction in the mPFC. These

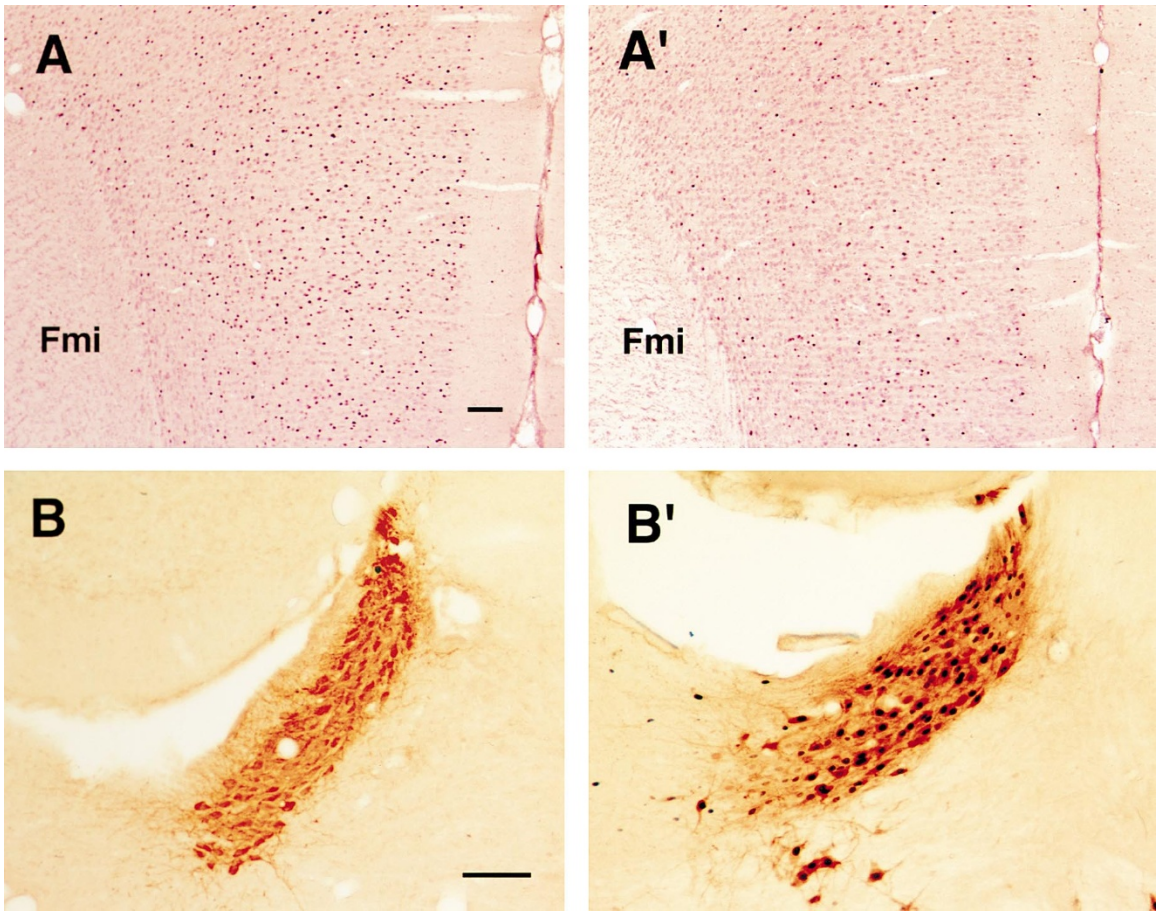

Figure 3. Photomicrographs of Fos-LI, A and A′, in the Cg3 region. A: saline + clozapine. A′: propranolol + clozapine. B and B′: Fos-LI (black nuclei) and stained somata (brown), representing tyrosine hydroxylase-LI in the LC. B: saline. B′: saline + clozapine. Fmi, forceps minor of corpus callosum. Bar = 100 μm.

results could be explained by up-regulation of β-adrenoceptors on the postsynaptic membrane acting as a compensatory mechanism after noradrenergic lesions (Cohen et al. 1985), resulting in no net change in Fos expression in the mPFC following clozapine administration.

Olanzapine, a clozapine-like atypical neuroleptic, possesses an affinity for various neurotransmitter receptors including α1-adrenoceptors; dopamine D1, D2, D3 and D4 receptors; 5-HT2A and 5-HT2C receptors; histaminergic receptors and muscarinic receptors (Bymaster et al. 1996a). It also increases noradrenaline release in the mPFC (Li et al. 1998; Westerink et al. 1998) and induces Fos expression preferentially in the mPFC (Robertson and Fibiger 1996; Sebens et al. 1998).

The present study demonstrated that olanzapine-induced Fos expression in the mPFC is also mediated by β-adrenoceptors. Because olanzapine also has a very low affinity for β-adrenoceptors (Bymaster et al. 1996a), olanzapine may increase noradrenaline release and thereby activate neurons in the mPFC via stimulation of the unblocked β-adrenoceptors. In contrast to clozapine, olanzapine possesses only weak α2-adrenoceptor antagonistic activity (Bymaster et al. 1996a,b), and therefore it is unclear how olanzapine induces Fos expression in the LC. Li and coworkers (1998) speculated that the mechanism of olanzapine-induced noradrenaline release may involve α1-adrenoceptor antagonistic activity, because olanzapine possesses the α1-adrenoceptor antagonistic activity as strong as that of clozapine. However, because prazosin, a potent α1-adrenoceptor antagonist, does not induce noradrenaline release in the mPFC (Gobert et al. 1998), this postulated α1-antagonistic mechanism does not seem to be the case. Further study is required to resolve this matter.

Propranolol is a non specific β-adrenoceptor antagonist and possesses 5-HT_{1A} antagonistic activity. So there is some possibility that either clozapine- or olanzapine-induced Fos expression in the mPFC is mediated by 5-HT_{1A} receptors. Olanzapine does not possess an affinity for 5-HT_{1A} receptors and does not induce 5-HT release in the mPFC (Bymaster et al. 1996a; Li et al. 1998), so the probability of 5-HT_{1A} receptor-mediated Fos expression is minimal. In contrast, clozapine possesses a weak 5-HT_{1A} agonistic activity (Bymaster et al. 1996a),

and Rollema et al. (1997) reported that clozapine affects the dopaminergic system in the mPFC via 5-HT_{1A} receptors. However, α2-adrenoceptor antagonistic activity is apparently stronger than 5-HT_{1A} agonistic activity (the Ki value is 8 nM for α2-adrenergic and 770 nM for 5-HT_{1A}) (Bymaster et al. 1996a). Thus, the 5-HT_{1A}-mediated mechanism is probably a minor effect, if it occurs at all.

The mPFC is rich in noradrenergic as well as dopaminergic and serotonergic nerve terminals (Parnavelas 1990). There is a large body of evidence to suggest that hypometabolism in the prefrontal cortex contributes to the negative symptoms of schizophrenia (Weinberger 1988; Weinberger et al. 1988; Wolkin et al. 1992). In addition, Van Kammen et al. (1986) has reported the occurrence of decreased noradrenaline and MHPG concentrations in the cerebrospinal fluid (CSF) of schizophrenic patients with negative symptoms. Therefore, these negative symptoms may be attributable to dysfunction of the prefrontal cortex, including hypofunction of the noradrenergic network (Rao and Moller 1994). To support this, idazoxan, a selective α2-antagonist, is reported to augment therapeutic effect of typical neuroleptics in treatment of schizophrenia (Litman et al. 1993, 1996).

In accordance with experimental results, clozapine treatment induces the up-regulation of noradrenaline turnover and marked increases in noradrenaline levels in both the CSF (Ackenheil 1989; Picker et al. 1992) and plasma (Picker et al. 1992; Breier et al. 1994) of human subjects. Taken together, these results suggest that clozapine and olanzapine increase noradrenaline release and thereby activate neurons in the mPFC via stimulation of the unblocked β-adrenoceptors which then ameliorates the negative symptoms of schizophrenia. However, further study is needed to refine this hypothesis.

ACKNOWLEDGMENTS

This work was supported by a Research Grant (No. 08770770) from the Japanese Ministry of Education in 1996 and a research grant from Zikei Hospital in 1999.

REFERENCES

Ackenheil M (1989): Clozapine: Pharmacokinetic investigation and biochemical effects in man. Psychopharmacology 99:S32–S37

Ader J P, Sebens J B, Korf J (1980): Central levels of noradrenaline, 3-methoxy-4-hydroxyphenylethyleneglycol and cyclic AMP in the rat after activation of locus coeruleus neurons: Influence of single and repeated neuroleptic treatment. Psychopharmacology 70:239–245

Beasley CM, Tollefson G, Tran P, Sattterlee W, Sanger T, Hamilton S (1996): Olanzapine versus placebo and haloperidol: Acute phase results of the North American double-blind trial. Neuropsychopharmacology 14:111–123

Bing G, Stone E A, Zhang Y, Filer D (1992): Immunohistochemical studies of noradrenergic-induced expression of c-fos in the rat CNS. Brain Res 592:57–62

Bolden C, Cusack B, Richelson E (1991): Clozapine is a potent and selective muscarinic antagonist at the five cloned human muscarinic acetylcholine receptors expressed in CHO-K1 cells. Eur J Pharmacol 192:205–206

Breier A, Buchanan RW, Waltrip RW II, Listwak S, Holmes C, Goldstein DS (1994): The effect of clozapine on plasma norepinephrine: Relationship to clinical efficacy. Neuropsychopharmacology 10:1–7

Bymaster FP, Calligaro DO, Falcone JF, Marsh RD, Moore NA, Tye NC, Seeman P, Wong DT (1996a): Radioreceptor binding profile of the atypical antipsychotic olanzapine. Neuropsychopharmacology 14:87–96

Bymaster FP, Hemrick-Luecke SK, Perry KW, Fuller RW (1996b): Neurochemical evidence for antagonism by olanzapine of dopamine, serotonin, α1-adrenergic and muscarinic receptors in vivo in rats. Psychopharmacology 124:87–94

Cedarbaum JM, Aghajanian GK (1977): Catecholamine receptors on locus coeruleus neurons: Pharmacological characterization. Eur J Pharmacol 44:375–385

Claghorn J, Honigfeld G, Abuzzahab FS, Steinbook AR, Tuason V, Klerman G (1987): The risks and benefits of clozapine versus chlorpromazine. J Clin Psychopharmacol 7:377–384

Cohen RM, Aulakh C, McLellan C, Murphy DL (1985): 6-Hydroxydopamine pretreatment effects on α- and β-adrenergic receptor adaptation to clorgyline. Naunyn-Schmiedeberg's Arch Pharmacol 329:158–161

Coward DM (1992): General pharmacology of clozapine. Br J Psychiatry 160(Suppl 17):5–11

Deutch AY, Duman RS (1996): The effects of antipsychotic drugs on Fos protein expression in the prefrontal cortex: Cellular localization and pharmacological characterization. Neuroscience 70:377–389

Dragunow M, Faull R (1989): The use of c-fos as a metabolic marker in neuronal pathway tracing. J Neurosci Meth 29:261–265

Fink-Jensen A, Kristensen P (1994): Effects of typical and atypical neuroleptics on Fos protein expression in the rat forebrain. Neurosci Lett 182:115–118

Fink-Jensen A, Ludvigsen T S, Korsgaard N (1995): The effect of clozapine on Fos protein immunoreactivity in the rat forebrain is not mimicked by the addition of α1-adrenergic or 5HT2 receptor blockade to haloperidol. Neurosci Lett 194:77–80

Freedman JE, Aghajanian GK (1984): Idazoxan (RX 781094) selectively antagonizes α2-adrenoceptors on rat central neurons. Eur J Pharmacol 105:265–272

Gobert A, Rivet JM, Audinot V, Newman-Tancredi A, Cistarelli L, Millan MJ (1998): Simultaneous quantification of serotonin, dopamine and noradrenaline levels in single frontal cortex dialysates of freely-moving rats reveals a complex pattern of reciprocal auto-and heteroreceptor-mediated control of release. Neuroscience 84:413–429

Grant SJ, Bittman K, Benno RH (1992): Both phasic sensory stimulation and tonic pharmacological activation increase Fos-like immunoreactivity in the rat locus coeruleus. Synapse 12:112–118

Guo N, Klitenic MA, Tham CS, Fibiger HC (1995): Receptor mechanisms mediating clozapine-induced c-fos expression in the forebrain. Neuroscience 65:747–756

Hamamura T, Lee Y, Fujiwara Y, Kuroda S (1997): Serotonin 1A receptor agonists induce Fos protein expression in the locus coeruleus of the conscious rat. Brain Res 759:156–159

Hamilton SH, Revicki DA, Genduso LA, Beasley CM (1998): Olanzapine versus placebo and haloperidol: Quality of life and efficacy results of the North American double-blind trial. Neuropsychopharmacology 18:41–49

Holes VR (1990): The anatomy and function of noradrenaline in the mammalian brain. In Heal DJ, Marsden CA (eds), The pharmacology of noradrenaline in the central nervous system. Oxford, Oxford University Press, pp 1–40

Kane J, Honigfeld G, Singer J, Meltzer H (1988): Clozapine for the treatment-resistant schizophrenic: A double-blind comparison with chlorpromazine. Arch Gen Psychiatry 45:789–796

Kurokawa K, Narita M, Koshiya K, Hidaka K, Ohmori J, Satoh K (1997): Effects of YM-43611, a novel dopamine D2-like receptor antagonist, on immediate early gene expression in the rat forebrain. Neuropsychopharmacology 17:27–33

L'Heureux R, Dennis T, Curet O, Scatton B (1986): Measurement of endogenous noradrenaline release in the rat cerebral cortex in vivo by transcortical dialysis: Effects of drugs affecting noradrenergic transmission. J Neurochem 46:1794–1801

Li X-M, Perry KW, Wong DT, Bymaster FP (1998): Olanzapine increases in vivo dopamine and norepinephrine release in rat prefrontal cortex, nucleus accumbens and striatum. Psychopharmacology 136:153–161

Litman RE, Hong WW, Weissman EM, Su T-P, Potter WZ, Pickar D (1993): Idazoxan, an α2 antagonist, augments fluphenazine in schizophrenic patients: A pilot study. J Clin Psychopharmacol 13:264–267

Litman RE, Su T-P, Potter WZ, Hong WW, Pickar D (1996): Idazoxan and response to typical neuroleptics in treatment-resistant schizophrenia, comparison with the atypical neuroleptic, clozapine. Br J Psychiatry 168:571–579

Meltzer HY, Matsubara S, Lee JC (1989): Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin 2 pKi values. J Pharmacol Exp Ther 251:238–246

Parnavelas JG (1990): Neurotransmitters in the cerebral cortex. In Uylings HBM, Van Eden CG, De Bruin JPC, Corner MA, Feenstra MGP (eds), The Prefrontal Cortex. Progress in Brain Research. Vol. 85. Amsterdam, Elsevier, pp 13–29

Paxinos G, Watson C (1986): The Rat Brain in Stereotaxic Coordinates. 2nd ed. Sydney, Academic Press

Picker D, Owen RR, Litman RE, Konicki PE, Gutierrez R, Rapaport MH (1992): Clinical and biologic response to clozapine in patients with schizophrenia: Crossover comparison with fluphenazine. Arch Gen Psychiatry 49:345–353

Ramirez OA, Wang RY (1986): Locus coeruleus norepinephrine-containing neurons: Effects produced by acute and subchronic treatment with antipsychotic drugs and amphetamine. Brain Res 362:165–170

Rao ML, Moller HJ (1994): Biochemical findings of negative symptoms in schizophrenia and their putative relevance to pharmacologic treatment. Neuropsychobiology 30:160–172

Robertson GS, Fibiger HC (1992): Neuroleptics increase c-fos expression in the forebrain: Contrasting effects of haloperidol and clozapine. Neuroscience 46:315–328

Robertson GS, Matsumura H, Fibiger HC (1994): Induction patterns of Fos-like immunoreactivity in the forebrain as predictors of atypical antipsychotic activity. J Pharmacol Exp Ther 271:1058–1066

Robertson GS, Fibiger HC (1996): Effects of olanzapine on regional c-fos expression in rat forebrain. Neuropsychopharmacology 14:105–110

Rollema H, Lu Y, Schmidt AW, Zorn SH (1997): Clozapine increases dopamine release in prefrontal cortex by 5-HT1A receptor activation. Eur J Pharmacol 338:R3–R5

Sagar SM, Sharp FR, Curran T (1988): Expression of c-fos protein in brain: Metabolic mapping at the cellular level. Science 240:1328–1331

Sebens JB, Koch T, Ter Horst GT, Korf J (1998): Olanzapine-induced Fos expression in the rat fore brain; cross-tolerance with haloperidol and clozapine. Eur J Pharmacol 353:13–21

Sharp FR, Sagar SM, Swanson RA (1993): Metabolic mapping with cellular resolution: c-fos vs. 2-deoxyglucose. Crit Rev Neurobiol 7:205–228

Sokoloff P, Giros B, Martres MP, Bouthenet ML, Schwartz JC (1990): Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. Nature 347:146–151

Stone EA, Zhang Y (1995): Adrenoceptor antagonists block c-fos response to stress in the mouse brain. Brain Res 694:279–286

Van Kammen DP, Van Kammen WB, Mann LS, Seppala T, Linnoila M (1986): Dopamine metabolism in the cerebrospinal fluid of drug-free schizophrenic patients with and without cortical atrophy. Arch Gen Psychiatry 43:978-983

Van Tol HHM, Bunzow JR, Guan HC, Sunahara RK, Seeman P, Niznik HB, Civelli O (1991): Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine. Nature 350:610–614

Van Veldhuizen MJ, Feenstra MG, Boer GJ (1994): Regional differences in the in vivo regulation of the extracellular levels of noradrenaline and its metabolites in rat brain. Brain Res 635:238–248

Wan W, Ennulat DJ, Cohen BM (1995): Acute administration of typical and atypical antipsychotic drugs induces distinctive patterns of Fos expression in the rat forebrain. Brain Res 688:95–104

Weinberger DR (1988): Schizophrenia and the frontal lobe. Trends Neurosci 8:367–370

Weinberger DR, Berman KF, Illowsky BP (1988): Physiologi-

cal dysfunction of dorsolateral prefrontal cortex in schizophrenia. Arch Gen Psychiatry 45:609–615

Westerink BHC, de Boer P, de Vries JB, Kruse CG, Long SK (1998): Antipsychotic drugs induce similar effects on the release of dopamine and noradrenaline in the medial prefrontal cortex of the rat brain. Eur J Pharmacol 361:27–33

Wolkin A, Sanfilipo M, Wolf A P, Angrist B, Brodie J D, Rotrosen J (1992): Negative symptoms and hypofrontality in chronic schizophrenia. Arch Gen Psychiatry 49:959–965