ELSEVIER

European Journal of Pharmacology 319 (1997) 379–383

ejp

Short communication

Noradrenaline and adrenaline are high affinity agonists at dopamine D_4 receptors

Adrian Newman-Tancredi *, Valérie Audinot-Bouchez, Alain Gobert, Mark J. Millan

Department of Psychopharmacology, Institut de Recherches Servier, 125 Chemin de Ronde, 78290 Croissy-sur-Seine (Paris), France

Received 12 December 1996; accepted 17 December 1996

Abstract

The activity of monoamine neurotransmitters was examined at dopamine D_4 receptors. In competition binding with [^{3}H]spiperone, noradrenaline and adrenaline exhibited a high affinity binding component (K_H = 12.1 nM and 5.0 nM ,respectively), similar to that of dopamine (K_H = 2.6 nM), whereas serotonin (5-hydroxytryptamine, 5-HT) and histamine had low affinity (K_i > 1000 nM). Noradrenaline and adrenaline acted as agonists at dopamine D_4 receptors, stimulating receptor-mediated [^{35}S]guanylyl-γ-thiotriphosphate ([^{35}S]GTPγS) binding (EC_{50} = 7.8 and 5.8 μM, respectively, versus 0.1 μM for dopamine). The dopamine D_4 receptor-selective ligand, 3-(4-[4-chlorophenyl]piperazin-1-yl)methyl-1*H*-pyrrolo[2,3*b*]-pyridine (L 745,870) and the dopaminergic antagonists, spiperone, haloperidol and clozapine, inhibited noradrenaline-stimulated [^{35}S]GTPγS binding whereas α_1-, α_2- and β-adrenoceptor antagonists did not. These results indicate that dopamine D_4 receptors are activated by noradrenaline and adrenaline, although at 50–100-fold higher concentrations than dopamine.

Keywords: Dopamine D_4 receptor; L 745,870; Clozapine; Noradrenaline; Adrenaline; [^{35}S]GTPγS

1. Introduction

The dopamine D_4 receptor is of interest for several reasons. First, it is localised in limbic structures associated with regulation of mood and cognition, such as cerebral cortex and hippocampus (Lahti et al., 1996; Mrzljak et al., 1996). Second, the atypical antipsychotic, clozapine, has significant affinity at the dopamine D_4 receptor (Van Tol et al., 1991) and, third, dopamine D_4-like receptor upregulation in postmortem schizophrenic brain has been observed by some researchers (Seeman et al., 1995; Murray et al., 1995) – although not by others (Reynolds, 1996). The dopamine D_4 receptor may therefore represent a novel target for the understanding of the mechanisms underlying psychosis. However, its precise physiological significance is yet to be clarified. Indeed, whilst dopamine D_4 receptors expressed in COS 7 (African green monkey kidney) cells were found to display high affinity for dopamine (Van Tol et al., 1991) some preliminary evidence suggests that dopaminergic receptors may also exhibit high affinity for other neurotransmitters (Odagaki et al., 1995; Van der Graaf et al., 1995; Lanau et al., 1995). The present study therefore investigated the relative affinities of monoamine neurotransmitters at recombinant human dopamine D_4 receptors. Two receptor isoforms ($D_{4.4}$ and $D_{4.2}$) and two heterologous expression systems (Chinese hamster ovary and Sf9 cells) were used. Further, we investigated the agonist activity of monoamines by their ability to induce dopamine D_4 receptor-mediated stimulation of [^{35}S]guanylyl-γ-thiotriphosphate ([^{35}S]GTPγS) binding. Finally, a range of antagonists, including the novel, selective, dopamine D_4 receptor antagonist, 3-(4-[4-chlorophenyl]piperazin-1-yl)methyl-1*H*-pyrrolo[2,3*b*]-pyridine (L 745,870; Kulagowski et al., 1996), was used to demonstrate the involvement of dopamine D_4 receptors versus other dopaminergic or adrenergic receptors. The results present evidence of a potent interaction of both noradrenaline and adrenaline, in addition to dopamine, at dopamine D_4 receptors.

2. Materials and methods

2.1. Competition binding at dopamine D_4 receptors

CHO-$D_{4.4}$, CHO-$D_{4.2}$ (Receptor Biology, Baltimore, MD, USA) or Sf9-$D_{4.2}$ (BioSignal, Montreal, Canada) cell

* Corresponding author. Tel.: (33-1) 4118-2446; Fax: (33-1) 4118-2470; e-mail: 101511,274@compuserve.com

membranes were incubated with [^{3}H]spiperone (0.5 nM) and competing ligands in buffer A (50 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$ and 0.1% (w/v) ascorbic acid) for 1 h at 22°C. Non-specific binding was defined using haloperidol (10 μM). Membranes of mouse A9L-D_2 cells (Receptor Biology) were incubated with [^{125}I]iodosulpride (0.1 nM) and competing ligands in buffer A supplemented with bovine serum albumin (0.2%, w/v). Non-specific binding was defined using raclopride (10 μM). Incubations were terminated by rapid filtration and binding isotherms were analysed by non-linear regression using the program PRISM (GraphPad). One-site and two-site fits were compared by *F*-test. Data are means ± S.E.M. of at least three experiments.

2.2. [^{35}S]GTPγS binding to CHO-h$D_{4.4}$ membranes

CHO-$D_{4.4}$ membranes (50 μg protein) were incubated (20 min, 22°C) with agonists/antagonists in a buffer containing 20 mM HEPES (pH 7.4), 3 μM GDP, 3 mM $MgSO_4$, 0.1 nM [^{35}S]GTPγS. Non-specific binding was defined with GTPγS (10 μM). The stability of adrenaline and noradrenaline was verified: they were incubated under experimental conditions and their concentration at the start and end of the incubation periods was determined by high-performance liquid chromatography using an improved separation protocol (Gobert et al., 1995). No change in their concentration was observed. L 745,870 was synthesised by Servier chemists. Other drugs were from commercial sources.

3. Results

3.1. Competition binding at dopamine D_4 receptors

The dopamine D_4 receptor antagonist, L 745,870, and the antagonists, spiperone, haloperidol and clozapine, inhibited [^{3}H]spiperone binding to dopamine $D_{4.4}$ receptors monophasically (pseudo-Hill coefficients, n_H, close to unity) with K_i values of 1.58 ± 0.64, 0.22 ± 0.02, 1.79 ± 0.67 and 62.8 ± 11.8 nM respectively. In contrast, noradrenaline, adrenaline and dopamine inhibited [^{3}H]spiperone binding to CHO-$D_{4.4}$ CHO-$D_{4.2}$ and Sf9-$D_{4.2}$ receptors biphasically (Fig. 1A) yielding K_H values for the high affinity components of the isotherms between 2.6 and 14.9 nM (Table 1). In contrast, dopamine D_2 receptors exhibited low affinity for noradrenaline and adrenaline with K_H values of 810 ± 190 nM and 730 ± 250 nM respectively. Competition binding experiments on dopamine $D_{4.4}$ receptors were also carried out in the presence of guanylylimidotriphosphate (GppNHp; 100 μM). Under these conditions, the K_H values of noradrenaline, adrenaline and dopamine were increased as follows: noradrenaline 188 ± 73 nM (Fig. 1B), adrenaline 75 ± 20 nM, dopamine 9.6 ± 2.8 nM. Serotonin (5-hydroxytryptamine, 5-HT) and histamine had low affinity at CHO-$D_{4.4}$ CHO-$D_{4.2}$ and Sf9-$D_{4.2}$ receptors (K_i > 1000 nM; Fig. 1A).

3.2. [^{35}S]GTPγS binding to CHO-h$D_{4.4}$ membranes

Dopamine stimulated specific [^{35}S]GTPγS binding to CHO-$D_{4.4}$ membranes from basal levels of 3590 ± 440

Table 1
Ligand binding affinities (inhibition constants, nM) at dopamine D_4 receptors

	(−)-Adrenaline	(−)-Noradrenaline	(±)-Noradrenaline	Dopamine
CHO-$D_{4.4}$				
K_H	5.0 ± 1.6	12.1 ± 5.1	14.9 ± 5.2	2.6 ± 0.9
K_L	850 ± 490	1130 ± 320	1970 ± 990	1430 ± 850
% High	61.2 ± 6.8	51.1 ± 5.7	53.0 ± 8.3	65.3 ± 5.1
n_H	0.44 ± 0.04	0.42 ± 0.07	0.41 ± 0.05	0.37 ± 0.06
CHO-$D_{4.2}$				
K_H	8.3 ± 3.3	7.5 ± 1.0	10.0 ± 1.4	4.0 ± 0.9
K_L	470 ± 120	1660 ± 800	2260 ± 1290	430 ± 80
% High	49.5 ± 2.3	53.0 ± 7.9	48.6 ± 2.6	56.2 ± 1.5
n_H	0.43 ± 0.04	0.39 ± 0.05	0.40 ± 0.07	0.44 ± 0.03
Sf9-$D_{4.2}$				
K_H	6.8 ± 3.3	10.1 ± 3.7	16.6 ± 3.1	3.7 ± 3.0
K_L	180 ± 60	320 ± 90	718 ± 162	290 ± 270
% High	46.9 ± 16.1	31.7 ± 2.3	49.3 ± 8.0	54.8 ± 9.3
n_H	0.62 ± 0.02	0.68 ± 0.03	0.69 ± 0.06	0.63 ± 0.04

Inhibition of [^{3}H]spiperone binding to human recombinant CHO-$D_{4.4}$, CHO-$D_{4.2}$ and Sf9-$D_{4.2}$ receptors. Isotherms, analysed by non-linear regression, fitted best to a two-site model (*F*-test, $P < 0.05$). Inhibition constants (nM) are shown for the high affinity (K_H) and the low affinity (K_L) components of the isotherms. % High = percentage of high affinity sites; n_H = pseudo-Hill coefficient. Data are means ± S.E.M. of at least three independent determinations.

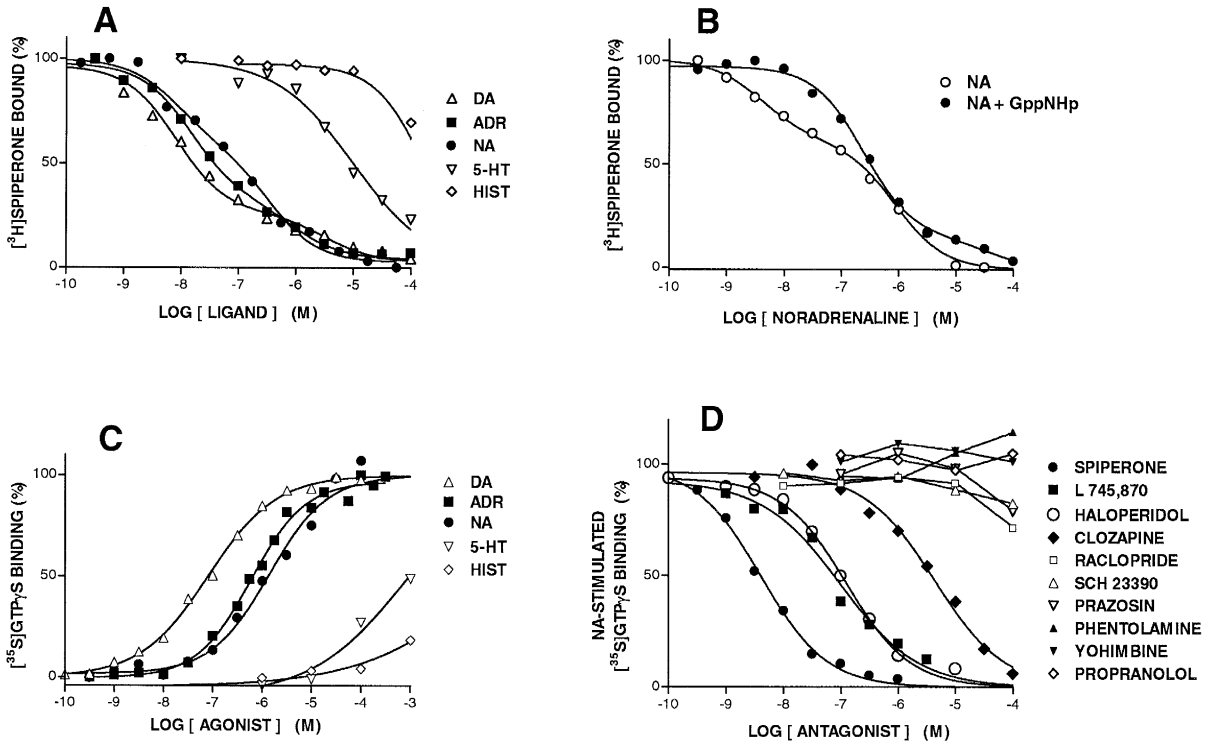

Fig. 1. Representative isotherms for (A) inhibition by adrenaline (ADR), (±)-noradrenaline (NA), dopamine (DA), serotonin (5-HT) and histamine (HIST) of [^{3}H]spiperone binding to human dopamine $D_{4.4}$ receptors; (B) inhibition by noradrenaline of [^{3}H]spiperone binding to human dopamine D_4 receptors in the presence and absence of GppNHp (100 μM); (C) stimulation of [^{35}S]GTPγS binding to CHO-$D_{4.4}$ cell membranes; (D) antagonism of noradrenaline (100 μM)-stimulated [^{35}S]GTPγS binding to CHO-$D_{4.4}$ cell membranes by dopaminergic and adrenergic antagonists.

dpm to a maximum of 8810 ± 480 dpm with an EC_{50} of 0.11 ± 0.01 μM. (±)-Noradrenaline and adrenaline also acted as agonists, with EC_{50} values of 7.8 ± 2.5 and 5.8 ± 1.6 μM respectively, whilst 5-HT and histamine only weakly stimulated [^{35}S]GTPγS binding ($EC_{50} > 0.1$ mM; Fig. 1C). Noradrenaline (100 μM)-stimulated [^{35}S]GTPγS binding was antagonised by spiperone (IC_{50} = 6.33 ± 1.31 nM), L 745,870 (IC_{50} = 46.5 ± 11.7 nM), haloperidol (IC_{50} = 70.6 ± 16.7 nM) and clozapine (IC_{50} = 4490 ± 1670 nM), which did not alter [^{35}S]GTPγS binding when tested alone. The pIC_{50} values for these compounds closely correlated with their pK_i values (r = 0.99, $P < 0.01$). Noradrenaline (100 μM)-stimulated [^{35}S]GTPγS binding was not ($IC_{50} > 10\,000$ nM) antagonised by raclopride, SCH 23390, prazosin, phentolamine, yohimbine or propranolol (Fig. 1D). No stimulation of [^{35}S]GTPγS binding by dopamine, adrenaline or noradrenaline was observed in membranes from untransfected CHO cells (n = 3, not shown).

4. Discussion

The present study shows that both noradrenaline and adrenaline, as well as dopamine, inhibited [^{3}H]spiperone binding to human recombinant CHO-$D_{4.4}$ receptors with high affinity (K_H = 12.1, 5.0 and 2.6 nM, respectively). These K_H values are similar to the affinitites of noradrenaline and adrenaline at α_1-, α_2- and β-adrenoceptors (Hieble et al., 1995). Nanomolar K_H values were also observed for a further dopamine D_4 receptor isoform ($D_{4.2}$) and in a different expression system (baculovirus-infected Sf9 cells), suggesting that high affinity for noradrenaline and adrenaline is an intrinsic characteristic of human dopamine D_4 receptors (Table 1). In contrast, recombinant human dopamine D_2 receptors exhibited low affinity for noradrenaline and adrenaline, indicating that high affinity for these neurotransmitters is not a general property of dopaminergic receptors.

The biphasic competition isotherms (Fig. 1A) suggest the presence of two G-protein-coupling states of the receptor. Indeed, an increase in K_H values was achieved in the presence of GppNHp, consistent with a change from a high agonist affinity (G-protein coupled) to a low agonist affinity (uncoupled) conformation of the receptor (Fig. 1B). In a functional test of intracellular signal transduction in CHO-$D_{4.4}$ membranes (activation of G-proteins as determined by stimulation of [^{35}S]GTPγS binding), noradrenaline and adrenaline, like dopamine, acted as agonists (Fig. 1C). It is unclear why noradrenaline and adrenaline are less potent than dopamine in stimulating [^{35}S]GTPγS binding. One possibility, which requires further investigation, is that they may promote coupling of the dopamine D_4 receptor to different G-protein populations, a process de-

noted 'agonist-receptor trafficking' (Kenakin, 1995). Thus, in comparison with dopamine, noradrenaline and adrenaline might induce coupling to a less 'tightly' coupled G-protein which is more sensitive to GppNHp. In any case, stimulation of [^{35}S]GTPγS binding by noradrenaline was specifically mediated by dopamine $D_{4.4}$ receptors, since the selective dopamine D_4 receptor antagonist, L 745,870 (Kulagowski et al., 1996), as well as spiperone, haloperidol and clozapine, completely antagonised it (Fig. 1C). In contrast, noradrenaline-induced [^{35}S]GTPγS binding was not inhibited by antagonists at dopamine D_2/D_3, D_1/D_5 receptors or α_1-, α_2- and β-adrenoceptors (Fig. 1D).

Previous studies have demonstrated interactions between signal transduction systems at the second messenger level, which may be activated by different receptor subtypes (Hadcock and Malbon, 1993), and at the neurotransmitter transporter level, e.g. the noradrenaline transporter also carries dopamine (Carboni et al., 1990). The present study now provides evidence that dopamine D_4 (but not D_2) receptors bind with high affinity, and are activated by, noradrenaline and adrenaline as well as dopamine. Although the present data were obtained using an in vitro model system, it raises several issues of potential physiological importance. First, dopamine D_4 receptor activation in vivo may be determined by the relative synaptic concentrations of noradrenaline, adrenaline and dopamine in different brain regions. For example, dopamine D_4 receptors are found in hippocampus and spinal cord (Lahti et al., 1996; Mrzljak et al., 1996; Matsumoto et al., 1996), which receive a major (nor)adrenergic but a sparse dopaminergic innervation (Moore and Bloom, 1979). Similarly, mRNA encoding dopamine D_4 receptors is detected in retina, adrenal chromaffin cells, heart and kidney (Matsumoto et al., 1995; Dahmer and Senogles, 1996), tissues rich in noradrenaline and adrenaline. Second, some studies associate increased noradrenergic activity with an intensification of the negative symptoms of schizophrenia and a susceptibility to relapse (Hornykiewicz, 1982; Van Kammen et al., 1990). These effects have classically been attributed to adrenoceptors, but it may be speculated that they might be mediated partly by noradrenergic activation of dopamine D_4 receptors.

In conclusion, the present study demonstrates that, in addition to dopamine, recombinant human dopamine $D_{4.4}$ and $D_{4.2}$ (but not D_2) receptors expressed in both CHO and Sf9 cells can potently bind adrenaline and noradrenaline. These act as agonists, stimulating dopamine D_4 receptor-mediated [^{35}S]GTPγS binding. These observations raise the possibility that dopamine D_4 receptors may mediate some of the physiological actions of noradrenaline and adrenaline. Further, some of the effects of the atypical antipsychotic, clozapine, which has relatively high affinity at dopamine D_4 versus other receptor subtypes, could be related to its blockade of noradrenergic, in addition to dopaminergic, transmission at dopamine D_4 receptors. However, confirmation that this can occur in vivo awaits the development of neurochemical and behavioural models of action at these sites.

Acknowledgements

We thank C. Chaput, L. Verrièle, V. Pasteau and S. Aubry for excellent technical assistance.

References

Carboni, E., G.L. Tanda, R. Frau and G. Di Chiara, 1990, Blockade of the noradrenaline carrier increases extracellular concentrations in the prefrontal cortex: evidence that dopamine is taken up in vivo by noradrenergic terminals, J. Neurochem. 55, 1067.

Dahmer, M.K. and S.E. Senogles, 1996, Dopaminergic inhibition of catecholamine secretion from chromaffin cells: evidence that inhibition is mediated by D_4 and D_5 dopamine receptors, J. Neurochem. 66, 222.

Gobert, A., J.M. Rivet, V. Audinot, L. Cistarelli, M. Spedding, J. Vian, J.-L. Peglion and M.J. Millan, 1995, Functional correlates of dopamine D_3 receptor activation in the rat in vivo and their modulation by the selective antagonist, (+)-S 14297: II. Both D_2 and 'silent' D_3 autoreceptors control synthesis and release in mesolimbic, mesocortical and nigrostriatal pathways, J. Pharmacol. Exp. Ther. 275, 899.

Hadcock, J.R. and C.C. Malbon, 1993, Agonist regulation of gene expression of adrenergic receptors and G-proteins, J. Neurochem. 60, 1.

Hieble, J.P., W.E. Bondinell and R.R. Ruffolo, 1995, α- and β-adrenoceptors: from the gene to the clinic. 1. Molecular biology and adrenoceptor subclassification, J. Med. Chem. 38, 3415.

Hornykiewicz, O., 1982, Brain catecholamines in schizophrenia – a good case for noradrenaline, Nature 299, 484.

Kenakin, T., 1995, Agonist-receptor efficacy. II: Agonist trafficking of receptor signals, Trends Pharmacol. Sci. 16, 232.

Kulagowski, J.J., H.B. Broughton, N.R. Curtis, I.M. Mawer, M.P. Ridgill, R. Baker, F. Emms, S.B. Freedman, R. Marwood, S. Patel, S. Patel, C.I. Ragan and P.D. Leeson, 1996, 3-[[4-(4-Chlorophenyl)piperazin-1-yl]-methyl]-1*H*-pyrrolo[2,3-b]pyridine: an antagonist with high affinity and selectivity for the human dopamine D_4 receptor, J. Med. Chem. 39, 1941.

Lanau, F., M.-T. Zenner, O. Civelli and D.S. Hartman, 1995, Epinephrine and norepinephrine act as potent agonists at the human recombinant $D_{4.4}$ receptor, Am. Soc. Neurosci. 21, 252.2.

Lahti, R.A., E.V. Cochrane, R.J. Primus, D.W. Gallager, R.C. Roberts and C.A. Tamminga, 1996, Dopamine D_4 receptor distribution in normal human postmortem brain sections: autoradiographic studies with [^{3}H]-NGD-94-1, Am. Soc. Neurosci. 22, 621.14.

Matsumoto, M., K. Hidaka, S. Tada, Y. Tasaki and T. Yamaguchi, 1995, Full-length cDNA cloning and distribution of human dopamine D_4 receptor, Mol. Brain Res. 29, 157.

Matsumoto, M., K. Hidaka, S. Tada, Y. Tasaki and T. Yamaguchi, 1996, Low levels of mRNA for dopamine D_4 receptor in human cerebral cortex and striatum, J. Neurochem. 66, 915.

Moore, R.Y. and F.E. Bloom, 1979, Central catecholamine neuron system: anatomy and physiology of the norepinephrine and epinephrine systems, Annu. Rev. Neurosci. 2, 113.

Mrzljak, L., C. Bergson, M. Pappy, R. Huff, R. Levenson and P.S. Goldman-Rakic, 1996, Localisation of dopamine D_4 receptors in GABAergic neurons of the primate brain, Nature 381, 245.

Murray, A.M., T.M. Hyde, M.B. Knable, M.M. Herman, L.B. Bigelow, J.M. Carter, D.R. Weinberger and J.E. Kleinman, 1995, Distribution of putative D_4 dopamine receptors in postmortem striatum from patients with schizophrenia, J. Neurosci. 15, 2186.

Odagaki, Y., S. Dasgupta and K. Fuxe, 1995, Additivity and non-additivity between dopamine-, norepinephrine-, carbachol- and GABA-stimulated GTPase activity, Eur. J. Pharmacol. 291, 245.

Reynolds, G.P., 1996, Dopamine D_4 receptors in schizophrenia? J. Neurochem. 66, 882.

Seeman, P., H.-C. Guan and H. Van Tol, 1995, Schizophrenia: elevation of dopamine D_4-like sites using [^{3}H]nemonapride and [^{125}I]epidepride, Eur. J. Pharmacol. 286, R3.

Van der Graaf, P.H., P.R. Saxena, N.P. Shankley and J.W. Black, 1995, Exposure and characterisation of the action of noradrenaline at dopamine receptors mediating endothelium-independent relaxation of rat isolated small mesenteric arteries, Br. J. Pharmacol. 116, 3237.

Van Kammen, D., J. Peters, J. Yao, W.B. Van Kammen, T. Neylan, D. Shaw and M. Linnoila, 1990, Norepinephrine in acute exacerbations of chronic scizophrenia, Arch. Gen. Psychiatry 47, 161.

Van Tol, H.H.M., J.R. Bunzow, H.-C. Guan, R.K. Sunahara, P. Seeman, H.B. Niznik and O. Civelli, 1991, Cloning of the gene for a human dopamine D_4 receptor with high affinity for the antipsychotic clozapine, Nature, 350, 610.