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Rapid communication

NGD 94-1 as an agonist at human recombinant dopamine $D_{4.4}$ receptors expressed in HEK293 cells

Lucien Gazi, Bernd Sommer, Joachim Nozulak, Philippe Schoeffter *

Nervous System Therapeutic Area, WSJ-386.7.44, Novartis Pharma Research, Novartis Pharma, CH-4002 Basel, Switzerland

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Abstract

The atypical antipsychotic, clozapine has some selectivity for dopamine D_4 receptors and is a silent antagonist at these receptors. NGD 94-1 (2-phenyl-4(5)-[4-(2-pyrimidinyl)-piperazin-1-yl-methyl]-imidazole) is a highly selective dopamine D_4 receptor ligand recently introduced as a putative antipsychotic mimicking the dopamine D_4 receptor antagonist effects of clozapine. We show that NGD 94-1 is not silent. It behaved as an agonist in human embryonic kidney 293 cells expressing human recombinant dopamine $D_{4.4}$ receptors. This questions the clinical use of NGD 94-1. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Dopamine D_4 receptor; NGD 94-1; Antipsychotic

The cloning of the gene coding for the dopamine D_4 receptor (a member of the dopamine D_2-like receptor family) has raised much interest in the neuropsychiatric field, because the atypical antipsychotic, clozapine shows some selectivity for it over classical dopamine D_2 receptors (Van Tol et al., 1991). Clozapine is devoid of many of the side effects (in particular, extrapyramidal side effects) associated with the use of typical antipsychotics. Recently, highly selective dopamine D_4 receptor ligands have been made available, with the intention to mimic the D_4 receptor antagonist effect of clozapine (see review by Wilson et al., 1998). One of them, L-745,870 (3-(4-[4-chlorophenyl]piperazin-1-yl)-methyl-1*H*-pyrrolo[2,3-b]pyridine) has entered clinical trials and proved to be ineffective in the treatment of psychosis, thereby questioning the D_4 receptor antagonist approach for treating schizophrenia (Kramer et al., 1997). However, we have found that L-745,870 behaved as an agonist with substantial efficacy at human recombinant dopamine D_4 receptors expressed in human embryonic kidney (HEK) 293 cells and negatively coupled to cyclic AMP (cAMP) formation (Gazi et al., 1998). In the same model, clozapine acted purely as an antagonist. This intriguing finding implies that L-745,870 might not have been the appropriate compound to conduct clinical antipsychotic trials. We therefore set out to investigate another selective D_4 receptor ligand, 2-phenyl-4(5)-[4-(2-pyrimidinyl)-piperazin-1-yl-methyl]-imidazole (NGD 94-1), also introduced as an antagonist (Tallman et al., 1997).

The functional activities of NGD 94-1 and dopamine were assessed by measuring drug-induced inhibition of forskolin-stimulated cAMP accumulation in intact HEK293 cells stably expressing the human dopamine $D_{4.4}$ receptor (HEK293/D_4 cells) and [^{3}H]spiperone binding was performed on cell homogenates as previously described (Gazi et al., 1998). NGD 94-1 was synthesized at Novartis Pharma. Data are given as means ± S.E.M. of the indicated n values.

Dopamine reduced the accumulation of cAMP induced by forskolin in HEK293/D_4 cells in a concentration-dependent manner (E_{max} 57 ± 3% inhibition, pEC_{50} 8.72 ± 0.13, $n = 7$; Fig. 1a). NGD 94-1 mimicked the effect of dopamine (E_{max} 45 ± 4% inhibition, pEC_{50} 8.94 ± 0.18, $n = 7$; Fig. 1a). The E_{max} of NGD 94-1 represented 79% of that of dopamine. NGD 94-1 did not inhibit forskolin-stimulated cAMP formation in control, non-transfected HEK293 cells (Fig. 1a). Spiperone (0.1 μM) shifted the concentration-response curve of NGD 94-1 to the right in a

* Corresponding author. Tel.: +41-61-324-92-61; Fax: +41-61-324-64-58; E-mail: philippe.schoeffter@pharma.novartis.com

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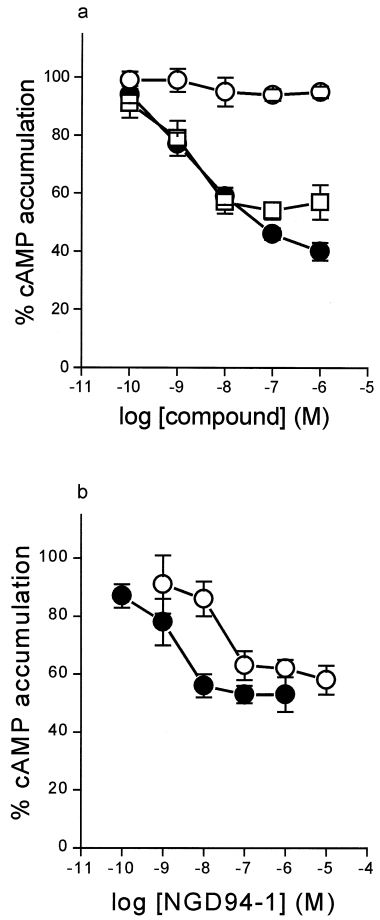

Fig. 1. Concentration–response curves for inhibition of forskolin-stimulated cAMP accumulation of (a) dopamine (●; $n = 7$) and NGD 94-1 (□; $n = 7$) in HEK293/D_4 cells and NGD 94-1 in non-transfected HEK293 cells (○; $n = 3$); (b) NGD 94-1 in the absence (●) and in the presence (○) of 0.1 μM spiperone in HEK293/D_4 cells ($n = 4$ each).

parallel manner (Fig. 1b). The derived pK_B value of spiperone was 8.31 ± 0.19 ($n = 4$). In competition binding experiments using HEK293/D_4 cell homogenates, NGD 94-1 inhibited the specific binding of [^{3}H]spiperone in a monophasic manner (pK_i 8.28 ± 0.11, $n = 2$).

The present study shows that NGD 94-1 acted as a dopamine D_4 receptor agonist in HEK293 cells stably expressing human dopamine $D_{4.4}$ receptors. NGD 94-1-induced inhibition of cAMP accumulation occurred in the nanomolar concentration range, compatible with the radioligand binding pK_i of the compound. Since the effects of NGD 94-1 were potently antagonised by spiperone and were not observed in non-transfected cells, there is little doubt that they are ascribed to dopamine D_4 receptor stimulation. The reason for which others (Tallman et al., 1997) could not see any agonist effect of NGD 94-1 is not clear. It might relate to the transfected cell type (Chinese hamster ovary vs. HEK293 cells) or the receptor variant used ($D_{4.2}$ vs. $D_{4.4}$). However, our results clearly indicate that under some circumstances NGD 94-1 can behave as a dopamine D_4 receptor agonist and may therefore not be the compound of choice to mimic the beneficial effects of clozapine in the clinic.

References

Gazi, L., Bobirnac, I., Danzeisen, M., Schüpbach, E., Bruinvels, A.T., Geisse, S., Sommer, B., Hoyer, D., Tricklebank, M., Schoeffter, P., 1998. The agonist activities of the putative antipsychotic agents, L-745,870 and U-101958 in HEK293 cells expressing the human dopamine $D_{4.4}$ receptor. Br. J. Pharmacol. 124, 889–896.

Kramer, M.S., Last, B., Getson, A., Reines, S.A., 1997. The effects of a selective D_4 dopamine receptor antagonist (L-745,870) in acutely psychotic inpatients with schizophrenia. Arch. Gen. Psychiatry 54, 567–572.

Tallman, J.F., Primus, R.J., Brodbeck, R., Cornfield, L., Meade, R., Woodruff, K., Ross, P., Thurkauf, A., Gallager, D.W., 1997. I. NGD 94-1: identification of a novel, high-affinity antagonist at the human dopamine D_4 receptor. J. Pharmacol. Exp. Ther. 282, 1011–1019.

Van Tol, H.H.M., Bunzow, J.R., Guan, H.-C., Sunahara, R.K., Seeman, P., Niznik, H.B., Civelli, O., 1991. Cloning of the gene for a human dopamine D_4 receptor with high affinity for the antipsychotic clozapine. Nature 350, 610–614.

Wilson, J.M., Sanyal, S., Van Tol, H.H.M., 1998. Dopamine D_2 and D_4 receptor ligands: relation to antipsychotic action. Eur. J. Pharmacol. 351, 273–286.