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Influence of the antipsychotic drug pipamperone on the expression of the dopamine D4 receptor

Kathleen Van Craenenbroeck [a,c], Evelien Gellynck [a], Béatrice Lintermans [a], Josée E. Leysen [b], Hubert H.M. Van Tol [c], Guy Haegeman [a], Peter Vanhoenacker [a,*]

[a] *Laboratory for Eukaryotic Gene Expression and Signal Transduction (LEGEST), Department of Molecular Biology, Ghent University - UGent, K.L. Ledeganckstraat 35, 9000 Gent, Belgium*
[b] *Department of Biochemical Pharmacology, Janssen Research Foundation, Turnhoutseweg 30, 2340 Beerse, Belgium*
[c] *Laboratory of Molecular Neurobiology – University of Toronto, Centre for Addiction and Mental Health – Clarke div., 250, College Street, Toronto, Ontario, Canada, M5T 1R8*

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Abstract

The dopamine D4 receptor is a G protein-coupled receptor that binds with high affinity various antipsychotics. The receptor may be involved in attention/cognition, and in genetic studies a polymorphic repeat sequence in its coding sequence has been associated with attention deficit/hyperactivity disorder. We developed an inducible episomal expression system based on the reverse tetracycline transactivator and Epstein-Barr viral sequences. In HEK293rtTA cells expressing the dopamine D4 receptor from this episomal expression vector, addition of doxycycline in combination with sodium butyrate and trichostatin A induces high levels of receptor expression, resulting in 1970±20 fmol/mg membrane protein. Addition of the dopamine D4 receptor and serotonin 5-HT2A receptor antagonist pipamperone to these cells further increased the expression of the dopamine receptor, reaching 3800±60 fmol/mg membrane protein. This up-regulation was not restricted to the dopamine D4 receptor but was also found for the serotonin 5-HT2A receptor. We further provide evidence that the increase in receptor expression is not due to increased mRNA synthesis. As pipamperone could rescue the expression of a folding mutant of the dopamine D4 receptor (M345), we propose that pipamperone acts as a pharmacological chaperone for correct receptor folding thereby resulting in an increased dopamine D4 receptor expression. This study describes a strong and inducible expression system for proteins, difficult to express in other heterologous expression systems. This study also demonstrates that pipamperone, an antipsychotic, acts as a pharmacological chaperone and by doing so, increases the expression level of the dopamine D4 receptor. The fact that ligands can also act as pharmacological chaperones is a fairly new additional element in the regulation of receptor expression levels with potential great impact in drug treatment.

Keywords: Inducible expression; Epstein-Barr virus; G protein-coupled receptor; Chaperone; Pipamperone; Dopamine D4 receptor

Introduction

G protein-coupled receptors (GPCRs) represent the largest family of signalling molecules in the human genome. They are characterized by a seven transmembrane alpha-helical structure and enable the cell to respond appropriately to particular extracellular stimuli. As more than 60% of all drugs are believed to interact with these receptors, the mechanisms by which the drugs mediate their interaction are of particular interest. The study of GPCRs is often hindered by problems with efficient expression of the active protein. We have previously described several heterologous systems for expression of GPCRs (Van Craenenbroeck et al., 2000a,b, 2001, 2003a; Vanhoenacker et al., 1997, 1999). Unlike non-mammalian high expression systems the main advantages of these mammalian systems are (1) that the cellular environment more closely resembles that of the native tissue in which GPCRs are expressed (e.g. presence of interacting and regulating proteins, comparable membrane lipid composition)

* Corresponding author. Tel.: +32 9 264 5135; fax: +32 9 264 5304.
E-mail address: Peter.vanhoenacker@ugent.be (P. Vanhoenacker).

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and (2) that the GPCRs show the correct post-translational modifications. Therefore these cells can be used to examine receptor signalling and regulation. Here we describe the use of the tet-on system (Gossen et al., 1995) in combination with an episomal Epstein-Barr virus (EBV)-derived vector (Van Craenenbroeck et al., 2000b, 2003a,b) for heterologous expression of the human dopamine D4 receptor (DRD4). We used this system to study the role of ligand-mediated up-regulation of receptor expression.

Dopamine is an important neurotransmitter of the central nervous system that participates in a variety of physiological functions. Its actions are mediated through interaction with five distinct dopamine receptors, which are all GPCRs. The dopamine receptor family can be subdivided in the DRD1 subfamily (DRD1 and DRD5), which couples to G_s and transduces the signal via activation of adenylyl cyclase, and the DRD2 subfamily (DRD2, DRD3 and DRD4), which couples to G_i resulting in inhibition of adenylyl cyclase and various other effectors. Dopamine signalling is of importance in motor control, reward and cognition. This system is the main target for therapeutic intervention in Parkinson's disease and schizophrenia, and is responsible for the addictive properties of drugs of abuse like amphetamine. For the DRD4 gene, mutations have been associated with various behavioural phenotypes, including attention deficit/hyperactivity disorder (Oak et al., 2000). This gene contains a polymorphic number (2–11 copies) of tandem 48 nucleotide repeats (Lichter et al., 1993; Van Tol et al., 1991, 1992; Wang et al., 2004), and is generally found to be difficult to express at high levels. In our expression system we succeeded to express this receptor at high levels. Pipamperone, an antipsychotic with strong DRD4 and 5-HT2A receptor (5-HT2AR) affinity (Ki 5.1 nM and Ki 5.4 nM, respectively) and moderate DRD2 affinity (Ki 110 nM) (Schotte et al., 1996), could further enhance the expression of the DRD4. Similar observations were made for the 5-HT2AR. The pipamperone-mediated up-regulation is likely mediated through a mechanism that involves stabilization of newly synthesized receptor in the native or intermediate state of its folding. Therefore, the main finding of this article is the fact that pipamperone can act as a pharmacological chaperone.

Materials and methods

Recombinant DNA constructions

The episomal EBV-derived vector, p220.2 (Yates et al., 1985), consists of the viral sequences oriP and EBNA1 and includes a hygromycin B selectable marker. For the construction of p220.2tetluc, the *Xho*I–*Bsr*BI fragment of pUHC13-3 (Gossen et al., 1995), consisting of TRE (tetracycline-responsive element)–CMV_{min}–luciferase–polyA, was inserted into the *Hind*III–*Sal*I site of p220.2. The vector ptetDRD4 resulted from the insertion of the *Asc*I–*Pac*I (blunt) fragment of DRD4 (Van Tol et al., 1992) into an *Xba*I-linearized, blunted and dephosphorylated pUHD10-3 vector (Gossen and Bujard, 1992). The vector p220.2tetDRD4 was constructed by inserting the *Ssp*I–*Hind*III fragment, containing TRE–CMV_{min}–DRD4–polyA, of ptetDRD4 into p220.2, opened with *Hind*III–*Hpa*I. Epitope tagged DRD4 expressing vectors have been described elsewhere (Van Craenenbroeck et al., 2005). The coding region of the 5-HT2AR was PCR-amplified and the cDNA was cloned behind a 1600 bp fragment of the murine Mx1 promoter (Hug et al., 1988) as described earlier (Vanhoenacker et al., 1997).

Cell culture and transfection

HEK293rtTA cells (human embryonic kidney cells expressing the tetracycline transactivator) (Vanhoenacker et al., 1999), L929sA pMx5-HT2AR cells (mouse fibrosarcoma; Rega Institute, Leuven, Belgium) (Vanhoenacker et al., 1997) and CHO cells (Chinese hamster ovary KI) were cultivated in a controlled environment (37 °C, 5% CO_2, 98% humidity) in Dulbecco's modified Eagle's medium (DMEM) (HEK293rtTA and L929sA) or minimal essential medium (CHO) supplemented with serum (10% newborn calf serum; 5% fetal calf serum–5% newborn calf serum or 10% fetal calf serum, respectively), L-glutamine (2 mM), penicillin (100 U/ml) and streptomycin (0.1 mg/ml). All stable transfections were carried out by standard calcium phosphate DNA coprecipitation procedures. Stable transfectants were selected by hygromycin B (150 U/ml for HEK293rtTA, Duchefa Biochemie, Haarlem, The Netherlands) over a period of 3 weeks. Stable clones, containing EBV-derived episomes, were kept under continuous hygromycin B selection to avoid a decrease of copy number (Yates et al., 1985). CHO cells stably expressing the DRD4.4 variants were described earlier (Oak et al., 2001; Van Craenenbroeck et al., 2005) and kept under continuous neomycine selection (500 μg/ml) (Invitrogen, San Diego, CA).

Induction–treatment

Cell cultures were grown to subconfluency. For induction of the tet-system, the medium was replaced by DMEM with 10% serum containing 1 μg/ml doxycycline (Duchefa Biochemie, Haarlem, The Netherlands), a tetracycline analogue. After an induction period of 24 h, cells were washed and lysates were made.

For induction of the Mx1 promoter the medium on L929sA cells, grown to subconfluency, was replaced by medium containing purified mouse interferon beta (1000 U/ml, home-produced in *E. coli*) for 24 h. The chemical agents brefeldin A (Sigma-Aldrich, St. Louis, MO) (5 μg/ml), sodium butyrate (Sigma-Aldrich) (2.5 mM) and trichostatin A (Biomol, Plymouth Meeting, PA) (100 nM) were added to the doxycycline-containing induction medium. The ligands pipamperone (Janssen Pharmaceutica, Beerse, Belgium) (100 nM), quinpirole (Sigma) (10 μM) and raclopride (Tocris Bioscience, Ellisville, MO) were added for 16 h or 24 h (as indicated).

Reporter protein quantification

Luciferase quantification was performed as described previously (Vanden Berghe et al., 1998). Light emission was