The following full text is a publisher's version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/16758

Please be advised that this information was generated on 2018-02-25 and may be subject to change.
Cloning and sequence analysis of a hypothalamic cDNA encoding a D_{1c} dopamine receptor in tilapia

Anne E. Lamers, Diet Gröneveld, Dominique P.V. de Kleijn, Felix C.G. Geeraedts, Jack A.M. Leunissen, Gert Flik, Sjoerd E. Wendelaar Bonga, Gerard J.M. Martens

Department of Animal Physiology, Faculty of Science, University of Nijmegen, Toernooiweg, NL-6525 ED Nijmegen, The Netherlands

CAOS / CAMM centre, University of Nijmegen, Toernooiweg, NL-6525 ED Nijmegen, The Netherlands

Received 3 January 1995; revised 9 April 1996; accepted 11 April 1996

Abstract

Physiological and pharmacological studies have indicated that during acid stress a D_{1c}-like dopamine receptor becomes functional on intermediate pituitary melanocyte-stimulating hormone cells of tilapia (Oreochromis mossambicus). As a first step towards physiological expression studies we isolated a D_{1c}-like dopamine receptor from a tilapia hypothalamus cDNA library. Construction of a phylogenetic tree of most of the D_{1c}-like receptors known in human, rat, Xenopus, goldfish and Drosophila revealed that the here presented clone is most likely the tilapia equivalent of the Xenopus D_{1c} dopamine receptor.

Keywords: Cloning; Sequence analysis; Dopamine receptor D_{1c}; (Hypothalamus); (O. mossambicus)

Dopamine signals are transduced via two types of dopamine receptors, D_{1}-like and D_{2}-like [1]. D_{1}-like receptors are coupled to a stimulatory G-protein to effect a stimulation of a second messenger system in the cell (see review Ref. [2]). D_{1}-like dopamine receptors are generally coupled to an inhibitory G-protein enabling the inhibition of a second messenger. The D_{1}-like and the D_{2}-like receptors can be distinguished pharmacologically using specific D_{1} and D_{2} agonists and antagonists. Receptor subclases of the D_{1}-like and D_{2}-like receptors have been identified at the molecular level: D_{2}-like receptors have been classified into D_{2}, D_{1a} and D_{1b}; D_{1}-like into the subtypes D_{1a}/D_{1b} and D_{1c}/D_{1b} [2,3]. Recently an additional D_{1}-like receptor subtype was found in the Xenopus, termed D_{1c} [4].

The regulation of release of α-melanocyte-stimulating hormone (α-MSH) from the pituitary neurointermediate lobe (NIL) of tilapia (Oreochromis mossambicus) during adaptation to acidified water has been described previously [5,6]. Pharmacological studies revealed that a D_{1}-like dopamine receptor activity was induced in the α-MSH-producing cells of the tilapia NIL when the fish were exposed for 7 days to acid water (pH 4.5) [7]. This receptor has a higher affinity for dopamine than the D_{2}-like receptor present in the NIL. The activation of these D_{1}-like and D_{2}-like receptors appears to result in stimulation of α-MSH release at picomolar concentrations of dopamine but in inhibition at nano- to micromolar dopamine concentrations. As a first step towards a study of dopamine receptor expression in the tilapia pituitary gland, we here describe the isolation and sequencing of a hypothalamic cDNA clone encoding a tilapia D_{1c}-like dopamine receptor.

Construction of the tilapia hypothalamic cDNA library.

A tilapia cDNA library was constructed from about 4 μg hypothalamic poly(A) RNA in EMBL Nucleotide Sequence Database accession number in EMBL Nucleotide Sequence Database: X81969.
Fig. 1. Nucleotide sequence and deduced amino acid sequence of hypothalamic cDNA clone pTDAl encoding the tilapia D2 dopamine receptor. Numbering starts at the putative initiation methionine and ends at the termination codon. The positions of the transmembrane (TM) regions are overlined.
An Uni-ZAP XR vector. The resulting library contained approx. $2 \times 10^5$ independent clones and was amplified according to standard procedures [10].

**Screening of the cDNA library.** Approx. 100,000 recombinants of the tilapia hypotalamic cDNA library were screened using a human $D_1$ dopamine receptor gene probe (hD,3z) [11]. The probe was $^{32}$P-labelled by random priming according to standard procedures [10]. After pre-hybridization in hybridization buffer containing 40 mM sodium phosphate-buffered (pH 7.4), 25% formamide, 6 $\times$ SSC [1 $\times$ SSC = 150 mM NaCl and 15 mM sodium citrate], 0.1% sodium dodecyl sulfate (SDS), 100 $\mu$g/ml denatured herring sperm DNA, 0.1% polyvinylpyrrolidone, 1 mM EDTA and 2 $\times$ Denhardt solution [1 $\times$ Denhardt solution is 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin and 0.1% Ficoll 400], filters were incubated with the probe in hybridization buffer at 42°C. After 18 h, filters were washed twice for 30 min with 2 $\times$ SSC at room temperature, 0.1% SDS and twice for 30 min with 2 $\times$ SSC, 0.1% SDS, at 56°C. Hybridization-positive clones were purified, and Bluescript DNA was prepared by in vivo excision according to the Stratagene protocol.

**DNA sequence analysis.** DNA sequencing of both strands was performed with T$_7$ DNA polymerase and the diodeoxy chain termination method [12], using subclones and synthetic oligonucleotides. Sequence alignments were performed according to Needleman and Wunsch [13] using the GAP program of the GCG program package [14].

**Construction of the phylogenetic tree.** The amino-acid sequence of the tilapia $D_1$-like receptor was aligned with the following dopamine receptors obtained from the databases SWISSPROT and EMBL: goldfish $D_1$ (gf$D_1$; accession No. P35406), human $D_1$ (h$D_1$; P21728), human $D_3$ (h$D_3$; P21918), rat $D_1$ (r$D_1$; P18901), rat $D_2$ (r$D_2$; P25115) the Xenopus $D_{la}$ (X$D_{la}$; X107863), Xenopus $D_{lb}$ (X$D_{lb}$; X107864), Xenopus $D_{lc}$ (X$D_{lc}$; X107865), and the translated Drosophila melanogaster $D_{1/5}$ (dm$D_{1/5}$; X77234). Invariant positions were deleted from the alignment, 1000 bootstrap samples were created using the SEQBOOT [15] program, and phylogenetic trees were obtained with the programs NEIGHBOR [16] and PROTPARS [17,18].

**Isolation and sequence analysis of hypothalamic cDNA encoding a tilapia $D_1$-like dopamine receptor.** Screening of approx. 100,000 recombinants of the amplified tilapia hypothalamic cDNA library resulted in the isolation of three hybridization-positive phage plaques. Restriction analysis revealed that the three clones contained the same 4.7 kb insert. One clone (pTDA1) was used for further analysis. Analysis of the nucleotide sequence of the pTDA1 clone revealed an open reading frame (ORF) coding for a protein of 368 amino acids (Fig. 1). Seven putative transmembrane regions, characteristic for G-protein coupled receptors, may be assigned to the protein. The clone contained an extremely long 5' non-coding region of approx. 2.2 kb and a 3' non-coding region of about 1.3 kb.

The deduced amino-acid sequence of pTDA1 showed high similarity to the $D_1$-like dopamine receptors (Table 1). The degree of amino-acid sequence identity between the putative G-protein coupled receptor and the human/rat $D_1/D_{la}$, the human/rat $D_1/D_{lb}$, and the Xenopus $D_{lc}$ receptors is about 66%, 65% and 74%, respectively. The amino-acid sequences of the two human stimulatory dopamine receptors ($D_1$ and $D_2$) show about 65% identity, whereas the stimulatory human $D_1$ and the inhibitory human $D_2$ receptor show only 29% identity [19]. From this we conclude that the tilapia clone encodes a stimulatory ($D_1$-like) dopamine receptor, most likely of the $D_{lc}$ subtype. We further term the here presented clone tilapia $D_{lc}$ ($D_{lc}$).

The 5' untranslated region of the $D_{lc}$ receptor contains several small ORFs of which the four most downstream are shown (Fig. 1). The first three ORFs consist of 66 bases and the fourth of 24 bases. Although small ORFs are generally rare in vertebrate mRNA, they are not uncommon in mRNAs of receptor genes, proto-oncogenes and growth-control genes [20,21]. The function of the small ORFs is yet unknown, but a role in the regulation of translational initiation of the main ORF has been suggested [22,23]. It is not clear whether the small upstream ORFs

| Amino-acid sequence identities in percentages, between the human $D_1$ (h$D_1$), rat $D_{la}$ (r$D_{la}$), Xenopus $D_{la}$, human $D_1$ (h$D_1$), rat $D_{lb}$ (r$D_{lb}$), Xenopus $D_{lb}$ (x$D_{lb}$), goldfish $D_1$ (gf$D_1$), Xenopus $D_{lc}$ (x$D_{lc}$), tilapia $D_{lc}$ (t$D_{lc}$) and Drosophila $D_{1/5}$ (dm$D_{1/5}$). |
|---------------------------------|----------------|----------------|--------------------|----------------|----------------|----------------|----------------|
| h$D_1$ | r$D_{la}$ | x$D_{la}$ | h$D_1$ | r$D_{la}$ | x$D_{la}$ | gf$D_1$ | x$D_{lc}$ | t$D_{lc}$ | dm$D_{1/5}$ |
| h$D_5$ | 100 | 87.3 | 75.5 | 63.4 | 62.8 | 64.5 | 65.7 | 68.3 | 65.0 | 38.2 |
| r$D_{lb}$ | 100 | 75.7 | 64.5 | 62.6 | 65.4 | 65.4 | 68.9 | 65.1 | 38.1 |
| x$D_{lb}$ | 100 | 65.7 | 64.9 | 67.9 | 68.3 | 71.3 | 64.6 | 39.3 |
| h$D_1$ | 100 | 90.4 | 83.2 | 76.3 | 68.6 | 65.9 | 37.2 |
| r$D_{la}$ | 100 | 100 | 82.7 | 76.0 | 67.4 | 65.8 | 36.4 |
| x$D_{la}$ | 100 | 78.6 | 69.9 | 67.6 | 38.0 |
| gf$D_1$ | 100 | 71.8 | 69.4 | 40.3 |
| x$D_{lc}$ | 100 | 74.2 | 40.8 |
| t$D_{lc}$ | 100 | 37.1 |
| dm$D_{1/5}$ | 100 | | | | | | | | |
Fig. 3. Phylogenetic tree of the D₁-like dopamine receptor family. Numbers in branches indicate the bootstrap values calculated with the PROTPARS program.

are translated, because none of the AUG triplets of the small ORFs are in a favourable context for translational initiation (A/GNNATGG is considered the ideal context for translational initiation [24]).

Comparison of the tD₁c receptor with D₁-like receptors of other species. Fig. 2 shows an alignment of the amino-acid sequences of human D₅, rat D₆b, Xenopus D₆b, human D₁, rat D₆a, Xenopus D₆a, goldfish D₁, Xenopus D₁c, and Drosophila melanogaster D₁/5. The highest degree of identity is located in the transmembrane regions, the first and second intracellular loops, the first extracellular loop and in the regions adjacent to the transmembrane regions VI and VII of the third intracellular loop and the cytoplasmic tail. The length of the cytoplasmic tail of the tD₁c/D₁ receptor is intermediate compared to that of the goldfish D₁ [25] and those of the mammalian D₆a and D₆b receptors and the amphibian D₁c receptor [4,19,26]. Presumably, this has no consequence for binding to the G-protein, as recent studies have revealed that only the region of the cytoplasmic loop nearest to the transmembrane VII is involved in G-protein coupling [27,28].

The percentages of overall sequence identity between the tD₁c dopamine receptors and hD₁, hD₅, rD₁a, rD₁b, xD₁a, XD₁b, xD₁c, gFD₁ and dmdD₁/5 receptors are shown in Table 1. The tD₁c receptor is related similarly the mammalian D₁/₁a and D₅/₁b receptors, and showed the highest identity (74.2%) with the recently discovered third D₁-like receptor of the Xenopus (XD₁c) [4]. Phylogenetic trees of members of the D₁-like dopamine receptor family were constructed to verify the identity of the tD₁c receptor. The topologies obtained by the NEIGHBOR and PROTPARS programs were essentially identical. The consensus tree of 1000 bootstrap samples of the PROTPARS program is shown in Fig. 3. The consensus tree indicates that the Drosophila D₁/5 receptor, the human D₁/rat D₁a/Xenopus D₁a/goldfish D₁ receptors, the human D₅/rat D₁b/Xenopus D₁b receptors and the Xenopus D₁c/tilapia D₁c are four significantly divergent groups within the family of D₁-like dopamine receptors (P = 1.00). The sequence of emergence of the three subtypes from the ancestral D₁ gene cannot be concluded from this tree.

References