Available online at www.sciencedirect.com
ScienceDirect
Insect Biochemistry and Molecular Biology 36 (2006) 749–758
Insect Biochemistry and Molecular Biology
www.elsevier.com/locate/ibmb

Identification and characterisation of the dopamine receptor II from the cat flea *Ctenocephalides felis* (CfDopRII)

Sonja Gerber[a], Andreas Krasky[a], Andreas Rohwer[a], Simone Lindauer[a], Ellen Closs[b], Didier Rognan[c], Nikolas Gunkel[a], Paul M. Selzer[a,*,1], Christian Wolf[a,*,2]

[a]*Intervet Innovation GmbH, Zur Propstei, 55270 Schwabenheim, Germany*
[b]*Department of Pharmacology, Johannes-Gutenberg-University, Obere Zahlbacher Straße 67, 55131 Mainz, Germany*
[c]*CNRS UMR 7175-LC1, Institut Gilbert-Laustriat 74, route du Rhin, B.P.24, F 67401 Illkirch, Germany*

Received 24 April 2006; received in revised form 12 July 2006; accepted 13 July 2006

Abstract

G protein-coupled receptors (GPCRs) represent a protein family with a wide range of functions. Approximately 30% of human drug targets are GPCRs, illustrating their pharmaceutical relevance. In contrast, the knowledge about invertebrate GPCRs is limited and is mainly restricted to model organisms like *Drosophila melanogaster* and *Caenorhabditis elegans*. Especially in ectoparasites like ticks and fleas, only few GPCRs are characterised. From the cat flea *Ctenocephalides felis*, a relevant parasite of cats and dogs, no GPCRs are known so far. Thus, we performed a bioinformatic analysis of available insect GPCR sequences from the honeybee *Apis mellifera*, the mosquito *Anopheles gambiae*, the fruit fly *Drosophila melanogaste*r and genomic sequences from insect species. Aim of this analysis was the identification of highly conserved GPCRs in order to clone orthologs of these candidates from *Ctenocephalides felis*. It was found that the dopamine receptor family revealed highest conservation levels and thus was chosen for further characterisation. In this work, the identification, full-length cloning and functional expression of the first GPCR from *Ctenocephalides felis*, the dopamine receptor II (CfDopRII), are described.
© 2006 Elsevier Ltd. All rights reserved.

Keywords: *Ctenocephalides felis*; G protein-coupled receptors; Dopamine receptors; *Drosophila melanogaster*; RNAinterference; Functional characterisation

1. Introduction

The cat flea *Ctenocephalides felis* is an ectoparasite with a life cycle comparable to that of *Drosophila melanogaster* which affects cats and dogs. Fleas are not restricted to one host, e.g. they can change their habitat from companion animals to humans and vice versa. Larvae feed on danders and dried blood excreted by the adult fleas. Flea bites can transmit infections or induce severe allergies (Dryden and Rust, 1994). The genome of the cat flea is not sequenced yet and only a small number of proteins (75 entries in Genbank, NCBI http://www.ncbi.nlm.nih.gov/Taxonomy) is already known. So far, no G protein-coupled receptors (GPCRs) from *Ctenocephalides felis* have been described.

GPCRs represent a protein family with up to 300 members in insects. They all share a common topology of seven transmembrane helices. GPCRs transduce extracellular signals into the intracellular cytoplasm (Brody and Cravchik, 2000). Heterotrimeric G proteins couple to GPCRs and affect different cell signaling pathways (Bissantz, 2003; Broeck, 2001). For instance, $G\alpha_s$ activates the adenylyl cyclase, whereas $G\alpha_i$ inhibits this enzyme (Neves et al., 2002). To facilitate identification of the first GPCR from *Ctenocephalides felis*, we decided to select a gene family of high conservation. Therefore, we examined the degree of similarity between insect GPCRs employing a bioinformatic analysis. This investigation revealed that dopamine receptors belong to the GPCR sub-family

*Corresponding author.
E-mail addresses: Paul.Selzer@Intervet.com (P.M. Selzer), Christian.Wolf@Intervet.com (C. Wolf).
[1]Tel.: +496130948206; fax: +496130948517.
[2]Tel.: +496130948366; fax: +496130948517.

0965-1748/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ibmb.2006.07.003

showing the highest degree of conservation. The three known dopamine receptors from *Drosophila melanogaster* (Dm) and *Apis mellifera* (Am) are divided into two classes: The DopRI and DopRII are functionally D1-like and activate the adenylyl cyclase (Genbank entries: AmDopRI/*Am*DOP1, CAA73841, Mustard et al., 2003; AmDopRII/*Am*DOP2, AAM19330, Mustard et al., 2003; DmDopRI/dDA1, AAA85716, Gotzes et al., 1994 and Sugamori et al., 1995; DmDopRII/DAMB/DopR99B, Q24563, Han et al., 1996 and Feng et al., 1996). In contrast, dopamine receptors III are related to D2-like receptors inhibiting the adenylyl cyclase (Genbank entries: AmDopRIII/*Am*DOP3,AY921573, Beggs et al., 2005; DmDopRIII/DD2R, AAN15955, Hearn et al., 2002). The pharmacology of invertebrate dopamine receptors was investigated in different expression systems: Dopamine receptors from *Drosophila melanogaster* were characterised in COS7- and Sf9-cells (DmDopRI, Sugamori et al., 1995), in *Xenopus laevis* oocytes (DmDopRII, Feng et al., 1996) and in HEK293 cells (DmDopRII, Han et al., 1996 and DmDopRIII, Hearn et al., 2002). AmDopRI was functionally expressed in HEK293 cells, Sf21- and Sf9-cells (Blenau et al., 1998; Mustard et al., 2003), AmDopRII in Sf21- and Sf9-cells (Humphries et al., 2003; Mustard et al., 2003) and AmDopRIII in HEK293 cells (Beggs et al., 2005). Moreover, GPCRs from *Anopheles gambiae* were predicted with bioinformatic tools: All three dopamine receptors, orthologs of the receptors from *Drosophila melanogaster* and *Apis mellifera,* have been identified in the genomic sequences from *Anopheles gambiae,* but these genes have not been cloned and characterised yet (Hill et al., 2002).

Here, we describe a bioinformatic analysis of conservation levels between insect GPCR sequences and the cloning of the first GPCR from the cat flea *Ctenocephalides felis*. The new dopamine receptor (CfDopRII) was functionally characterised by the expression in HEK293 cells and in *Xenopus laevis* oocytes. Moreover, the in vivo function of the receptor was investigated in the model organism *Drosophila melanogaster* by RNAi gene knock-down.

2. Materials and methods

2.1. Bioinformatic analysis

The genome comparison tool Genlight (Beckstette et al., 2004) was used for the analysis of different sequence sets. GPCR sequences from *Drosophila melanogaster* were obtained from FlyBase (batch download from http://flybase.bio.indiana.edu/; Brody and Cravchik, 2000). Additionally, GPCR sequences from *Anopheles gambiae* (Hill et al., 2002) and the genomic sequence data from *Aedes aegypti* (www.ensemble.org; Aedes_aegypti. AEDES1.august.pep_tigr.fa and Aedes_aegypti.AEDES1.august.pep_vector base.fa), *Anopheles gambiae* (www.ensemble.org; Anopheles_gambiae.A gamP3-feb.pep.fa), *Apis mellifera* (www.ensemble.org; Apis_mellifera.AMEL 2.0.feb.pep.fa), *Bombyx mori* (http://silkworm.genomics.org.cn; SW_ge2k_BGF.pep) and from *Drosophila melanogaster* (www.ensemble.org; Drosophila_melanogaster.BDGP4.feb.pep.fa) were used.

Predictions of transmembrane helices were performed with TMHMM server version 2.0 (www.cbs.dtu.dk/services/TMHMM/; Krogh et al., 2001). Alignments were created with ClustalW version 1.82 (www.ebi.ac.uk/clustalw/; Thompson et al., 1994) and edited in GeneDoc version 2.6.001 (www.psc.edu/biomed/genedoc/; Nicholas et al., 1997). BLAST searches with single sequences were accomplished at NCBI (http://www.ncbi.nlm.nih.gov/BLAST/; Altschul et al.,1990). The phylogenetic tree was created with the software Metalife Trinity 2006 version 2.2, Metalife AG, Winden, Germany (www.metalife.de/index.html). Identities between different sequences were determined with Vector NTI Advance version 9.0, Invitrogen, Carlsbad, USA; scoring matrix BLOSUM62.

2.2. Design of degenerate primers and cloning of CfDopRII

Degenerate primers were designed for highly conserved regions with a low level of degeneracy (Kwok et al., 1994). The degenerate primers (first PCR forward primer: CAYACIGCNACIAAYTAYTT and first PCR reverse primer: CCARCAIGCRTADATIACIGGRTTCAT; second PCR forward primer: ATGCCITTYWSIGCIYTNTA and second PCR reverse primer: TTDATCCAICCIARCCANGT) were used for nested PCR to amplify gene fragments from cDNA which was derived from reverse transcription of RNA from *Ctenocephalides felis*.

The sequence of the fragments was determined and the degree of similarity with other insect dopamine receptors was investigated using BLAST searches. After identification of a first fragment, the 5′- and 3′-ends of the gene were cloned by nested RACE-PCR (BD SMART RACE cDNA Amplification Kit, BD Biosciences, Franklin Lakes, NJ, USA; first PCR 3′-end: TCCTATCTGGGTTGTGCGTGAATTGTATCC; second PCR 3′-end: AACACGAAGAAATAGTATCGGCGGTAGTCA; first PCR 5′-end: AACAAGACATCCAGGGAGCGCCAAATGTCA; second PCR 5′-end: CACCAGTCGGAGCCAAAGAACCAAGTGTTG). The open reading frame for CfDopRII was predicted with Vector NTI Advance version 9.0 (Invitrogen, Carlsbad, USA). Afterwards the full length open reading frame was amplified from cDNA of *Ctenocephalides felis* and optimized according to the Kozak sequence upstream of the start codon (Kozak, 1987). The primers for the amplification contained an *Eco*RI and *Bgl*II overhang for suitable cloning (forward primer: GAATTCAGATCTGCCATGAATATCAGTTTCAAC; reverse primer: GAATTCAGATCTATGCTAAATGTAGGATTGTCCATAG). The CfDopRII was cloned into the vector pCRIITOPO (Invitrogen, Carlsbad, USA). The sequences of four independent clones were determined in order to obtain a sequence free of PCR errors. For expression in *Xenopus laevis* oocytes CfDopRII was inserted in the *Bgl*II site of pSP64 T (Krieg and

Download English Version:

https://daneshyari.com/en/article/1983162

Download Persian Version:

https://daneshyari.com/article/1983162

Daneshyari.com