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The Endocannabinoid System in the Baboon (*Papio SPP.*) as a Complex Framework for Developmental Pharmacology

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Abstract

Introduction—The consumption of marijuana (exogenous cannabinoid) almost doubled in adults during last decade. Consumption of exogenous cannabinoids interferes with the endogenous cannabinoid (or “endocannabinoid” (eCB)) system (ECS), which comprises *N*-arachidonylethanolamide (anandamide, AEA), 2-arachidonoyl glycerol (2-AG), endocannabinoid receptors (cannabinoid receptors 1 and 2 (CB1R and CB2R), encoded by *CNR1* and *CNR2*, respectively), and synthesizing/degrading enzymes (FAAH, fatty-acid amide hydrolase; MAGL, monoacylglycerol lipase; DAGL- α , diacylglycerol lipase- α). Reports regarding the toxic and therapeutic effects of pharmacological compounds targeting the ECS are sometimes contradictory. This may be caused by the fact that structure of the eCBs varies in the species studied.

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Objectives—First: to clone and characterize the cDNAs of selected members of ECS in a non-human primate (baboon, *Papio* spp.), and second: to compare those cDNA sequences to known human structural variants (single nucleotide polymorphisms and haplotypes).

Materials and methods—Polymerase chain reaction-amplified gene products from baboon tissues were transformed into *Escherichia coli*. Amplicon-positive clones were sequenced, and the obtained sequences were conceptually translated into amino-acid sequences using the genetic code.

Results—Among the ECS members, *CNR1* was the best conserved gene between humans and baboons. The phenotypes associated with mutations in the untranslated regions of this gene in humans have not been described in baboons. One difference in the structure of *CNR2* between humans and baboons was detected in the region with the only known clinically relevant polymorphism in a human receptor. All of the differences in the amino-acid structure of DAGL- α between humans and baboons were located in the hydroxylase domain, close to phosphorylation sites. None of the differences in the amino-acid structure of MAGL observed between baboons and humans were located in the area critical for enzyme function.

Conclusion—The evaluation of the data, obtained in non-human primate model of cannabis-related developmental exposure should take into consideration possible evolutionary-determined species-specific differences in the CB1R expression, CB2R transduction pathway, and FAAH and DAGL α substrate-enzyme interactions.

Keywords

Non-human primates; development; endocannabinoid system; homology; pharmacology

1. Introduction

Marijuana use has doubled among U.S. adults during the past decade (Hasin et al. 2015). Alarming, cannabinoids are the substance chiefly abused by pregnant women; its prevalence in this group exceeds 10% in the United States (Anonymous 2015; Alpar et al. 2015; Metz and Stickrath 2015). Prenatal exposure to the active component of marijuana, THC (Δ^9 -tetrahydrocannabinol), is associated with negative behavioral outcomes and psychopathology in offspring (Alpar et al. 2015; El Marroun et al. 2015). THC acts through the mechanism of “kick-starting” the components of the endogenous cannabinoid system (McPartland et al. 2015). The main ligands of the endogenous cannabinoid, or “endocannabinoid” (eCB), system (ECS) are anandamide (AEA), and 2-arachidonoyl glycerol (2-AG), which mediate cannabinimimetic effects (Smith et al. 1994). ECS includes endocannabinoid receptors (cannabinoid receptors 1 and 2 [CB1R and CB2R]), encoded by *CNR1* and *CNR2*, respectively); and synthesizing/degrading enzymes such as fatty-acid amide hydrolase (FAAH), monoacylglycerol lipase, (MAGL), and diacylglycerol lipase- α (DAGL α). Recently-identified novel eCB ligands and receptors have extended this family of lipid derivatives (Maccarrone et al. 2015). Modern discoveries regarding the role of endocannabinoids in the regulation of brain (Maccarrone 2005) and adipose tissue metabolism (You et al. 2011), pancreatic beta cell fate (Jourdan et al. 2013), pregnancy maintenance (Sun and Dey 2012), and cardiovascular disorders (Montecucco and Di Marzo

2012) have indicated that this system is one of the central players in mechanisms that are shared between metabolic and mental health disorders (Nousen et al. 2013), however reports regarding the therapeutic and toxic effects of cannabinoid and endocannabinoid derivatives are sometimes contradictory (Pacher and Hasko 2008) (Pacher et al. 2008; Pacher and Kunos 2013). The reason for these discrepancies may reside in the structural and functional eCB variants present in different models and organisms studied (McPartland et al. 2007a; Zhang et al. 2015). Specifically, in preclinical studies, a deep understanding of the genetics and physiology of animal models is required for predicting drug effects and toxicology (Vickers et al. 2011).

The variety of animal models used in cannabinoid and endocannabinoid research, including rodents (Marco et al. 2013), ruminants (Turco et al. 2008), and primates (Ames et al. 1979; Brocato et al. 2013; Edery 1983), has brought into question the comparative translational aspects of the results obtained in each model. Baboons (*Papio spp.*) are well-characterized Old World non-human primates that are used to study psychiatric (Goodwin et al. 2013), developmental, and pregnancy-related questions (Mari et al. 2014; Nathanielsz et al. 2015; Schlambitz-Loutsevitch et al. 2007), aging (McFarlane et al. 2011), as well as cardiovascular, and metabolic disturbances (Bommineni et al. 2011); (Comuzzie et al. 2003; Hurwitz and Rosendorff 1985); (see (Cox et al. 2013) for a review). While CB1R-mediated effects of THC and of CB1R inverse agonist/antagonist (Rimonabant) were similar in adult non-pregnant baboons and in humans (Ames et al. 1979; Charalambous et al. 1991; Levett et al. 1977; Meldrum et al. 1974; Vaidyanathan et al. 2012), the association between mental status and Rimonabant administration has not been described in baboons (Christensen et al. 2007; Zador et al. 2015), underlining the importance of understanding species-specific translational phenotypes. Surprisingly, there is no available structural information for the *ECS* gene family or “endocannabinoidome” (Di Marzo and Piscitelli 2015; Witkamp 2015) in *Papio spp.*

Thus the goal of this study was to clone and characterize the cDNAs of the ECS in baboons and to align them with known structural gene variants found in humans. We choose to study central receptors involved in the exogenous and endogenous cannabinoid effects (CB1R and CB2R) and the main enzymes regulating eCB tone (Petrosino and Di Marzo 2010). The data obtained should provide important information for translational pharmacological studies of substance abuse and development of new medications targeting ECS.

2. Materials and Methods

2.1. Animals

Tissues (liver) were collected during necropsies from animals undergoing pathological examination at the Southwest National Primate Research Center, Texas Biomedical Research Institute (San Antonio, TX, USA) and available to the investigators through the tissue share program. All animal procedures were approved by the Institutional Animal Care and Use Committee and were conducted within facilities approved by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

2.2. RNA extraction and retrotranscription reactions

Total RNA was extracted from the tissue samples using TRIZOL reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). The RNA was then treated with RQ1 DNase (Promega, Madison, WI, USA) for 15 minutes at 37°C to remove traces of genomic DNA. To assess RNA purity and integrity, we used standard methods of spectrophotometry and gel electrophoresis, respectively. The retro-transcription (RT) reactions were carried out using a High Capacity cDNA Reverse Transcription kit with 1 µg of total RNA, following the manufacturer's instructions (Applied Biosystems, Grand Island, NY, USA) and employing random primers (hexamers) acquired from Invitrogen (Carlsbad, CA, USA).

2.3. End-point polymerase chain reaction (PCR)

Primers for end-point PCR were designed using the Primer3 online tool (<http://frodo.wi.mit.edu/>) and other previously reported primate sequences found in GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>). Polymerase chain reaction (PCR) assays were carried out in a 25-µl total volume using 1 µl of each primer (10 pM), 5 µl of the RT reaction and 2X PCR Master Mix from Promega (San Luis Obispo, CA, USA). Amplification was carried out in a Veriti thermocycler (Applied Biosystems, Foster City, CA, USA) under the conditions described in Table 1 (supplementary material).

2.4. Molecular cloning, sequencing, and sequence analysis

The amplified products were cloned into the 3.5 kb-XL-TOPO vector and transformed into electrocompetent *Escherichia coli* bacteria of the strain Top 10 according to the manufacturer's specifications (Invitrogen, Life technologies, Grand Island, NY, USA). Positive clones were sequenced using the BigDye Terminator Cycle Sequencing Kit v3.1 with specific primers and/or M13 universal primers. The reactions were analyzed in an ABI PRISM 3100 Genetic Analyzer using Sequencing Analysis Software v5.3 (Applied Biosystems, Foster City, CA, USA). Sequencing chromatograms were edited for quality and trimmed to remove nonspecific sequences and primer sequences using the GeneStudio Pro software suite (GeneStudio, Inc., Suwanee, GA, USA).

The obtained sequences were conceptually translated using the Transeq online program and aligned with orthologous human genes using ClustalW (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>) followed by manual corrections where necessary (used in preparation of all figures). Both the nucleotide and amino-acid sequences were deposited in the GenBank database. From the coding sequences open reading frames (ORFs) were predicted, and both were aligned with orthologous human proteins to determine the percentage homology.

3. Results

3.1. CNR1

The ORF of the *CNR1* gene in baboon consists of 1419 nucleotides encoding 472 amino acids (aa) residues. Baboon *CNR1* shows 98% similarity in its nucleotide sequence and 100% similarity in its amino acid sequence to the orthologous human gene. The secondary structure includes two turns (340-344 and 394-401) and helices (378-391 and 403-410).

Protein structure has an extracellular N-terminus (1-116), three extracellular loops (176-187, 256-273, and 366-377), three intracellular loops (143-154, 213-232, and 300-344), one intracellular C-terminus (400-472), and seven transmembrane domains (117-142, 155-175, 188-212, 233-255, 274-299, 345-365, and 378-399) (Fig. 1).

3.2. CNR2

The *CNR2* ORF in baboons comprises 1,083 base pairs (bp), encoding 360 amino-acid residues. Baboon *CNR2* shows 95% homology in its nucleotide sequence and 96% in its amino-acid sequence to the orthologous human gene (Fig. 2). The predicted secondary structure has two beta strands (242-245 and 258-260), turns (246-254 and 261-265), and helices (255-257 and 266-269). Protein structure includes four extracellular domains (1-33, 93-104, 173-188 and 268-279), four cytoplasmic segments (60-71, 130-149, 215-246, and 302-360), and seven transmembrane domains (34-59, 72-92, 105-129, 150-172, 189-214, 247-267, and 280-301).

FAAH—The *FAAH* nucleotide sequence in baboons consists of 1740 bp and encodes an ORF of 579 aa. Baboon *FAAH* shows 96% homology in its nucleotide sequence and 96% homology in amino-acid sequence with the orthologous human gene. The protein structure includes one intramembrane domain (404–433), two cytoplasmic segments (30–403 and 434–579), one helical motif (9–29), and one substrate-binding site (238–241) (Fig. 3).

3.3. DAGL- α

The *DAGL- α* ORF in baboons consists of 3129 bp and encodes 1042 aa residues. Baboon *DAGL- α* shows 97% similarity with the orthologous human gene and 98% homology in the amino-acid structure. The structure includes two extracellular domains (44-60 and 123-136), three cytoplasmic segments (1-22, 82-101 and 158-1042), and four helical motifs (23-43, 61-81, 102-122, and 137-157) (Fig.4).

3.4. MGLL (MAGL)

The *MAGL* nucleotide sequence in baboons consists of 942 bp and encodes an ORF of 313 aa, exhibiting 97% homology in its nucleotide sequence and 98% homology in its amino-acid sequence with the orthologous human gene. The secondary structure includes ten beta strands (21-23, 29-35, 42-38, 70-75, 116-121, 140-146, 148-151, 231-236, 240-242, and 257-264), one turn (136-138), and eleven helices (16-18, 59-67, 94-110, 123-134, 153-168, 181-183, 188-195, 207-223, 244-253, 271-273, and 276-292) (Fig.5).

4. Discussion

4.1. Endocannabinoid receptors

Both types of endocannabinoid receptors belong to the family of G-protein coupled seven transmembrane receptors, and are called cannabinoid 1 receptor (CB1R, encoded by *CNR1*) and cannabinoid 2 receptor (CB2R, encoded by *CNR2*). The genetic structure of these receptors was described excellently by Onaivi et al. (Onaivi et al. 2002). The structure of given CB receptor determines specific interactions with orthosteric and allosteric ligands,

whereas allosteric interactions were shown to elicit species- and tissue-specific CB signaling (Cawston et al. 2013).

4.1.1. CNR1—CB1R, a target of exogenous and endogenous cannabinoids (Vemuri and Makriyannis 2015), was discovered over 3 decades ago (Matsuda et al. 1990; Munro et al. 1993). The most comprehensive sequencing of *CNR1* conducted to date was performed by Murphy *et al.* (Murphy et al. 2001) in 62 species, including *Macaca mulatta* (rhesus macaque) and *Homo sapiens* (human). Interestingly, there is no available information regarding the sequence of this receptor in the Old World non-human primate (order Cercopithecoidea), baboon (*Papio* spp.), which diverged from *Macaca* spp. approximately 9.8 mega annum (Ma). The cercopithecoid lineage in turn separated from the anthropoids (which gave rise to humans) approximately 23 Ma (Raaum et al. 2005). Fetal brain, retinal development and the central organ of pregnancy, the placenta, have differences and similarities across these three closely related primate lineages (Bouskila et al. 2016; Burton et al. 2015; Carter 2012; Rodríguez-Sánchez et al. 2013) and should be taken in consideration in analyses of species-specific cannabinoid effects (Bouskila et al. 2016). Our data are in agreement with the published observation that *CNR1* became a stabilized, conserved, gene in primate lineage (McPartland et al. 2007b). The effects of the selective CB1R antagonist (SR141716) are very similar in baboons and humans (Foltin and Haney 2007; Vaidyanathan et al. 2012), and CB1R radioligand binding, demonstrated in the baboon brain, is consistent with the binding observed in humans (Gatley et al. 1998). However, the association between mental status and SR141716 administration has been demonstrated only in humans and rats (Christensen et al. 2007; Zador et al. 2015), highlighting the importance of understanding species-specific translational phenotypes (Ogawa and Vallender 2014).

In the presence of the highly conserved structure of CB1R (Onaivi et al. 2002), evolutionary pressure has targeted mechanisms associated with the regulation of function and expression of the receptor (encoded in the untranslated region (UTR) (Juhász et al. 2009), rather than its amino-acid and 3D structures (encoded in the ORF) (McPartland et al. 2007b). For example, increased CB1R expression has been reported in pregnancy-related disorder –pre-eclampsia, described in anthropoids, but not in the baboons (Fugedi et al. 2014) and the CB1R density in cortex and amygdala is increased in human's, compared to brain of *Macaca mulatta* (Herkenham et al. 1990). In humans variations in the (UTR) of *CNR1* are associated preeclampsia (Bienertova-Vasku et al. 2011), autism-spectrum disorders (Chakrabarti and Baron-Cohen 2011), and high neuroticism and a low agreeableness phenotype (Juhász et al. 2009), which are conditions that have not been described in *Papio spp.* (Ogawa and Vallender 2014). The research, involving prenatal CB1R targeted exposure should take in consideration evolutionary differences in receptors expression.

4.1.2. CNR2—The CB2R has been identified in cells of the immune system, including neuroglia, macrophages, and brain endothelial cells (Persidsky et al. 2015), as well as in the placenta and endometrium (Brocato et al. 2013; Taylor et al. 2010). The CB2R structure is less conserved than that of CB1R, and the two receptors share only 44% structural similarity (Munro et al. 1993; Onaivi et al. 2002). Sequence analysis of the coding region of rat *CNR2* indicated 90% nucleic acid identity (93% amino-acid identity) between the rat and mouse

receptors and an 81% nucleic acid identity between the rat and human receptors (Griffin et al. 2000), which is lower than the 95-96% similarity between humans and baboons found in the present study. This difference could be important, as responses to CB2R receptor stimulation differ even among rodents (Zhang et al. 2015). The difference in the amino-acid sequences observed between baboons and humans is located in codon 63 (Q to R) (intracellular loop one). In humans, this codon is associated with polymorphism, and linked to auto-immune diseases (Sipe et al. 2005), depression (Onaivi et al. 2008), celiac disease (rs35761398), (Rossi et al. 2012), immune thrombocytopenic purpura (Mahmoud Gouda and Mohamed Kamel 2013; Rossi et al. 2011; Rossi et al. 2012), hepatonecrosis (Coppola et al. 2014), and age of menarche (Bellini et al. 2015). Interestingly, the differences in the amino-acid sequence observed between humans and baboons in our study are mostly located in the C-terminus of CB2R (Fig. 4), which may be responsible for signal transduction (Dhopeswarkar and Mackie 2014) but is not critical for receptor binding (Feng et al. 2014). The possible differences in signal transduction pathway might influence immunomodulatory effects of marijuana and others drugs targeting CB2R in non-human primate model.

4.2. FAAH

FAAH is the primary protein responsible for degradation in ECS signaling pathway (Bari et al. 2006; Deutsch et al. 2002; Fezza et al. 2008) and is located on chromosome 1 1p35-p34) in humans (Parsons and Hurd 2015). Interestingly, Mileni et al (Mileni et al. 2008) reported that six of active-site amino acids residues differed between rat (L192, F194, A377, S435, I491, and V495) and human FAAH (F192, Y194, T377, N435, V491, and M495), while in our study all these aa were identical between humans and baboons. Remarkably, one of the amino-acid changes observed in the baboon sequence was an I238M substitution. This amino-acid corresponds to part of the α -19 transversal helix, which constitutes the putative membrane-binding cap (Bracey et al. 2002; Palermo et al. 2015b) and is also part of the oxyanion hole, which maintains the substrate in the proper orientation for hydrolysis (Palermo et al. 2015a). This change might result in baboon-specific differences in FAAH function, similar to those described between rodents and humans (Mileni et al. 2008). In general the aa composition of rat FAAH had 82% homology with structure of the human enzyme compared to 96% homology found in the present study.

4.4. DAGL (α and β)

DAGL α and β (di-acylglycerol lipases, which are responsible for the synthesis of 2-AG) play important roles in neurodevelopment and adult neurogenesis (Oudin et al. 2011; Tejera et al. 2012), and their structure is conserved among various species (Bisogno et al. 2003). DAGLs also exhibit functions that do not involve cannabinoid receptors, such as producing intermediate products for the pathway and maintaining the levels of essential lipids in the brain and other organs (Reisenberg et al. 2012). The structure of DAGLs includes four transmembrane helices, extracellular domains and a canonical α/β hydrolase domain with catalytic activity, which is followed by a carboxyl-terminal 'tail' in DAGL- α (Reisenberg et al. 2012). The evolutionary history of DAGL- α correlates closely to that of CB1R (McPartland et al. 2007c), in agreement with this statement our study found a high (98%) similarity between the baboon and human amino-acid sequences of DAGL- α (CBR1 had 100% homology). All of the differences in the amino-acid structures were located in the

hydroxylase domain. The regions of this domain are close to the phosphorylation sites, which are important for the regulation of substrate access (Reisenberg et al. 2012).

4.5.MAGL (MGLL)

MAGL is the central molecule in 2-AG degradation and is responsible for 85% of 2-AG hydrolysis in the brain, connecting endocannabinoid metabolism to neuro-inflammation (Nomura et al. 2011; Savinainen et al. 2012). MAGL is essential for lipolysis, the cancer metabolic switch, and pain (reviewed in (Savinainen et al. 2012). This gene is located on Chr 3q21 in humans and Chr 6 in mice. The cysteine residues (C208 and C242) are critical for enzyme inhibition (Saario et al. 2005), Cys242 or Tyr194 mutations compromise hydrolysis and enzyme activity in vitro (Laitinen et al. 2014). None of the differences in the amino-acid structures identified between baboons and humans were located in the regions, critical for interaction with the substrate (Afzal et al. 2014). This gene is highly conserved across different species.

5. Conclusion

The evaluation of the data obtained in non-human primate model of cannabis-related developmental exposure should take into consideration possible evolutionary determined species-specific differences in the CB1R expression, CB2R transduction pathway, and FAAH and DAGL α substrate-enzyme interactions. The data presented herein provides important information for translational, toxicological, and pharmacological studies of exogenous and endogenous cannabinoids.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- The analyses of cDNA sequence of ECS in non-human primates are performed.
- The ECS sequences in baboons are compared to human sequences.
- The CNR1 has 100% and CNR2- 96% amino acids homology with human receptors.

Ph-CNR1	MKSILDGLADTTFRITITDLLYVGSNDIQYEDIKGDMAKSLGYFPQKFPLTSFRGSPFQE	60
Hs-CNR1	MKSILDGLADTTFRITITDLLYVGSNDIQYEDIKGDMAKSLGYFPQKFPLTSFRGSPFQE	60

Ph-CNR1	KMTAGDNPQLVPADQVNITEFYNKSLSSEFKENEENIQCGENFMDIECFMVLNPSQQLAIA	120
Hs-CNR1	KMTAGDNPQLVPADQVNITEFYNKSLSSEFKENEENIQCGENFMDIECFMVLNPSQQLAIA	120

Ph-CNR1	VLSTLTGFTVLENLLVLCVILHSRSLRCRPSYHFISGLAVADLLGSVIFVYSFIDFHFV	180
Hs-CNR1	VLSTLTGFTVLENLLVLCVILHSRSLRCRPSYHFISGLAVADLLGSVIFVYSFIDFHFV	180

Ph-CNR1	HRKDSRNVFLFKLGGVTASFTASVGSFLTAIDRYISIHRLPLAYKRIVTRPKAVVAFCLM	240
Hs-CNR1	HRKDSRNVFLFKLGGVTASFTASVGSFLTAIDRYISIHRLPLAYKRIVTRPKAVVAFCLM	240

Ph-CNR1	WTIAIVIAVLPLLGW N CEKLQSVCS D IFPHIDE TYLMFWIGVTSVLLLFIVYAYMYILWK	300
Hs-CNR1	WTIAIVIAVLPLLGW N CEKLQSVCS D IFPHIDE TYLMFWIGVTSVLLLFIVYAYMYILWK	300

Ph-CNR1	AHSHAVRMIQRGTQKSI I IHTSEDGKVQVTRPDQARMDIRLAKTLVLILVVLII C WGPLL	360
Hs-CNR1	AHSHAVRMIQRGTQKSI I IHTSEDGKVQVTRPDQARMDIRLAKTLVLILVVLII C WGPLL	360

Ph-CNR1	AIMVYDVFGK M NKLIKTVFAFC S MLCLLNS TVNPIIYALRSKDLRHAFRSMF P SCEGTAQ	420
Hs-CNR1	AIMVYDVFGK M NKLIKTVFAFC S MLCLLNS TVNPIIYALRSKDLRHAFRSMF P SCEGTAQ	420

Ph-CNR1	PLDNSMGDS D CLHKKHANNAASVHRAAESCIKSTVKIAKVTMSVSTDTSAEAL	472
Hs-CNR1	PLDNSMGDS D CLHKKHANNAASVHRAAESCIKSTVKIAKVTMSVSTDTSAEAL	472

Figure 1. Comparison of predicted CNR1-ORF from the baboon (*Papio anubis*) and human (*Homo sapiens*)

Human *CNR1* nucleotide sequence Gene Bank Gene NP_001153698 In bold: extracellular motif; In italics: helical domain; Underlined: cytoplasmic domain. Ph: *Papio spp*, Hs: *Homo sapiens*. The differences in amino acids composition between the two species are marked by rectangles.

Ph-CNR2 **MEECWVTEIANGSKNGLD FNPMDYMI LSGPQKIA IAVLCTLLGLLSALENVAVLYLILS** 60
Hs-CNR2 **MEECWVTEIANGSKDGLD SNPMKDYMI LSGPQKTAVAVLCTLLGLLSALENVAVLYLILS** 60

Ph-CNR2 SHRLERKPSYLF**FIGSLAGADFLASVVFACSFVNFHVFGMD**SKA VFLKIGSVTMTFTAS 120
Hs-CNR2 SHQLERKPSYLF**FIGSLAGADFLASVVFACSFVNFHVFGVD**SKA VFLKIGSVTMTFTAS 120

Ph-CNR2 VGSLLLTAIDRYLCLRYPPSYKALLTRGRALVTLGIMWVLSALVSYLPLMGW**TCCPRPCS** 180
Hs-CNR2 VGSLLLTAIDRYLCLRYPPSYKALLTRGRALVTLGIMWVLSALVSYLPLMGW**TCCPRPCS** 180

Ph-CNR2 **ELFPLIPNDYLLSWLLFIAFLFSGIIYTYGHVLWKAHQHVASLSGHQDRQVPGMARMRLD** 240
Hs-CNR2 **ELFPLIPNDYLLSWLLFIAFLFSGIIYTYGHVLWKAHQHVASLSGHQDRQVPGMARMRLD** 240

Ph-CNR2 VRLAKTLGLVLAVLLICWFPVLALN**HSLATTLSDQVKKAFAFCSMLCL**INSMVNPVIYA 300
Hs-CNR2 VRLAKTLGLVLAVLLICWFPVLALN**HSLATTLSDQVKKAFAFCSMLCL**INSMVNPVIYA 300

Ph-CNR2 LRSGEIRSSAHHCLAR**KKCVRGLGSEAKEEAPRSSVTET**EADGKITPWPD**SRDLI**SSDC 360
Hs-CNR2 LRSGEIRSSAHHCLAR**KKCVRGLGSEAKEEAPRSSVTET**EADGKITPWPD**SRDLI**SSDC 360

Figure 2. Comparison of predicted CNR2-ORF from the baboon (*Papio anubis*) and human (*Homo sapiens*)

Human *CNR2* nucleotide sequence Gene Bank Gene ID AJ430063.1 In bold: extracellular motif; In italics: helical domain; Underlined: cytoplasmic domain. Ph: *Papio spp*, Hs: *Homo sapiens*. The differences in amino acids composition between the two species are marked by rectangles.

Ph-FAAH MGQYELWAALPGASGVALACCFVAAAVLRWSGRRARGAVRARQRQACLENMDKAAQ 60
Hs-FAAH MVQYELWAALPGASGVALACCFVAAAVLRWSGRRRTARGAVRARQRQACLENMDRAAQ 60

Ph-FAAH RFRLQNPDLSEALLALPLPQLVQKLNGETSP EAVLFITYVGKAWENVKGTNCVTSYLAD 120
Hs-FAAH RFRLQNPDLSEALLALPLPQLVQKLH SREIAPEAVLFITYVGKAWENVKGTNCVTSYLAD 120

Ph-FAAH CETQLSQAPRQGLLYGVPVSLKECFTYKQDSTLGLSLNECAPAECDSVVVHVLKLQGA 180
Hs-FAAH CETQLSQAPRQGLLYGVPVSLKECFTYKQDSTLGLSLNECVPAECDSVVVHVLKLQGA 180

Ph-FAAH PFVHTNVPQSMFSYDCSNPLFGQTVNPKSSKSPGGSSGGEGALIGSGGSPLGLGTHMSG 240
Hs-FAAH PFVHTNVPQSMFSYDCSNPLFGQTVNPKSSKSPGGSSGGEGALIGSGGSPLGLGTHMSG 240

Ph-FAAH SIRFPSSFCGICGLKPTGNRLSKSGLKGCYVQGEAVF SVGPMARDVESLALCLRALLCE 300
Hs-FAAH SIRFPSSFCGICGLKPTGNRLSKSGLKGCYVQGEAVF SVGPMARDVESLALCLRALLCE 300

Ph-FAAH DMFRLDPTVPPLPFREHYTSSQPLRVGYETDNYTMTSPAMKRAVLETKQSLEAAGHTL 360
Hs-FAAH DMFRLDPTVPPLPFREHYTSSQPLRVGYETDNYTMTSPAMKRAVLETKQSLEAAGHTL 360

Ph-FAAH VPFLPSNIPHALETLS TGGLFSDGGHTFLQNFKGDFVLSLGLDLVSILKLPQWLKGLLAF 420
Hs-FAAH VPFLPSNIPHALETLS TGGLFSDGGHTFLQNFKGDFVLSLGLDLVSILKLPQWLKGLLAF 420

Ph-FAAH LVKPLLPRLSAFLSNMKSRSAGKLWELQHEIEVYRKTIVIAQWRALDLDVLTPLMIGPALD 480
Hs-FAAH LVKPLLPRLSAFLSNMKSRSAGKLWELQHEIEVYRKTIVIAQWRALDLDVLTPLMIGPALD 480

Ph-FAAH LNAPQRATGAVSYTMLYNCLDFPAGVVPVTTVTAEDEAQMEHYRGYFGDIWDIMLQKGMK 540
Hs-FAAH LNAPQRATGAVSYTMLYNCLDFPAGVVPVTTVTAEDEAQMEHYRGYFGDIWDIMLQKGMK 540

Ph-FAAH NSVGLPVAVQCVALPWQEELCLRFMREVERLMTPEKQSS 579
Hs-FAAH KSVGLPVAVQCVALPWQEELCLRFMREVERLMTPEKQSS 579

Figure 3. Comparison of predicted DAGLA-ORF from the baboon (*Papio anubis*) and human (*Homo sapiens*)

Human *DAGLA* nucleotide sequence Gene Bank accession number is: NM_006133.2 In bold: cytoplasmic; in italics: helical domain; underlined: extracellular. Ph: *Papio spp*, Hs: *Homo sapiens*. The differences in amino acids composition between the two species are marked by rectangles.

Ph-DAGLA	MPGIWFRRRWSVGSDDLVLPALFLFLHTTWVFLSWLFLGLVYNPHEACSLNLDVHGR	60
Hs-DAGLA	MPGIWFRRRWSVGSDDLVLPALFLFLHTTWVFLSWLFLGLVYNPHEACSLNLDVHGR	60
Ph-DAGLA	GYLGILLSCMIAEMAIWLSMRGGILYTEPRDSMQVVLVRLAILVIEFTYAVIGIVWLT	120
Hs-DAGLA	GYLGILLSCMIAEMAIWLSMRGGILYTEPRDSMQVVLVRLAILVIEFTYAVIGIVWLT	120
Ph-DAGLA	QYITSCNDLTAKNVILGMVVCNWWVILSVCIIVLCVFDPTGRTFVKLRATKRQRNLRTY	180
Hs-DAGLA	QYITSCNDLTAKNVILGMVVCNWWVILSVCIIVLCVFDPTGRTFVKLRATKRQRNLRTY	180
Ph-DAGLA	NLRHRLEEGQATSWSRRLKVF LCCTRTKDSQSDAYSEIAYLFAEFFRDLIVPSDIIAGL	240
Hs-DAGLA	NLRHRLEEGQATSWSRRLKVF LCCTRTKDSQSDAYSEIAYLFAEFFRDLIVPSDIIAGL	240
Ph-DAGLA	VLLRQRQRAKRNAVLDEANNDI LAF LSGMPVTRNTKYLD LKNSQEMLRKYKEVCYMLFAL	300
Hs-DAGLA	VLLRQRQRAKRNAVLDEANNDI LAF LSGMPVTRNTKYLD LKNSQEMLRKYKEVCYMLFAL	300
Ph-DAGLA	AAYGWPMYLMRKPAACGLCQLARSCSCCLCPARPRFAPGVITIEDNCCGCNAIAIRRHFLD	360
Hs-DAGLA	AAYGWPMYLMRKPAACGLCQLARSCSCCLCPARPRFAPGVITIEDNCCGCNAIAIRRHFLD	360
Ph-DAGLA	ENMTAVDIVYTSCHDAVYETPFYVAVDHDKKKVVISIRGTLSPKDALTDLTGDAERLPVE	420
Hs-DAGLA	ENMTAVDIVYTSCHDAVYETPFYVAVDHDKKKVVISIRGTLSPKDALTDLTGDAERLPVE	420
Ph-DAGLA	GHGCTWLGHKGMVLSAEYIKKKLEQEMVLSQAFGRDLGRGTHYGLIVVGHSLGAGTAAI	480
Hs-DAGLA	GHGCTWLGHKGMVLSAEYIKKKLEQEMVLSQAFGRDLGRGTHYGLIVVGHSLGAGTAAI	480
Ph-DAGLA	LSFLLRPQVPTLKCFAYSPPGGLLSEDA MEYSKEFVTAVVLGQDLVPRIGLSQLEGFRQ	540
Hs-DAGLA	LSFLLRPQVPTLKCFAYSPPGGLLSEDA MEYSKEFVTAVVLGQDLVPRIGLSQLEGFRQ	540
Ph-DAGLA	LLDVLQRSTKPKWRIIVGATKCI PKSELPEEVEVTTLASTRLWTHPSD LTIASASTPLY	600
Hs-DAGLA	LLDVLQRSTKPKWRIIVGATKCI PKSELPEEVEVTTLASTRLWTHPSD LTIASASTPLY	600
Ph-DAGLA	PPGRIIHVVHNHPAEQCCCEQEEPTFYFAIWGDNKAFNEVIISPAMLHEHLPYVMEGLN	660
Hs-DAGLA	PPGRIIHVVHNHPAEQCCCEQEEPTFYFAIWGDNKAFNEVIISPAMLHEHLPYVMEGLN	660
Ph-DAGLA	KVLENYNKGKTALLSAAKMVSPTVDLTPELIFQQQPLPTGPPMAGLALELPTADHRN	720
Hs-DAGLA	KVLENYNKGKTALLSAAKMVSPTVDLTPELIFQQQPLPTGPPMAGLALELPTADHRN	720
Ph-DAGLA	SSVRSKSSQSEMSLEGFSEGRLLSPVVAANAARODPVELLLLSTOERLAAELQARRAPLAT	780
Hs-DAGLA	SSVRSKSSQSEMSLEGFSEGRLLSPVVAANAARODPVELLLLSTOERLAAELQARRAPLAT	779
Ph-DAGLA	MESLSDTESLYSFDSSRSSGFRSIRGSPSLHAVLERDEGHLFYIDPAIPEENPSLSSRTE	840
Hs-DAGLA	MESLSDTESLYSFDSSRSSGFRSIRGSPSLHAVLERDEGHLFYIDPAIPEENPSLSSRTE	839
Ph-DAGLA	LLAADSLSKHSQDTQPLEVALGSGGVTPERPSSAANDEEIVGGGGGGLASRGE LALHN	899
Hs-DAGLA	LLAADSLSKHSQDTQPLEVALGSGGVTPERPSSAANDEEIVGGGGGGLASRGE LALHN	899
Ph-DAGLA	GRLGDSPPSPQVLEFAEFIDSLFNLD SKSSSFQDLYCMVVPESPTSDYAECPKSPSQEIL	959
Hs-DAGLA	GRLGDSPPSPQVLEFAEFIDSLFNLD SKSSSFQDLYCMVVPESPTSDYAECPKSPSQEIL	959
Ph-DAGLA	LRAQFEPNLVPKPPRLFAGSADPSGSLSPSFLSSSGLMDLTPTGLSSQEC LAADKI	1019
Hs-DAGLA	LRAQFEPNLVPKPPRLFAGSADPSGSLSPSFLSSSGLMDLTPTGLSSQEC LAADKI	1019
Ph-DAGLA	RTSTPTGHGASPAKQDELVISAR	1042
Hs-DAGLA	RTSTPTGHGASPAKQDELVISAR	1042

Figure 4. Comparison of predicted MGLL-ORF from the baboon (*Papio anubis*) and human (*Homo sapiens*)

Human *MGLL* nucleotide sequence Gene Bank accession number is: CR456835.1 In bold: turn; in italics: beta strand; underlined: helix. Ph: *Papio spp*, Hs: *Homo sapiens*. The differences in amino acids composition between the two species are marked by rectangles.

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Ph-MGLL      METQSEDPSRMPEESSPRRT PQSIPYQDLPHLVNADGQYLFCRYWKPTGTPKALIFVSHG 60
Hs-MGLL      METQSEDPSRMPEESSPRRT PQSIPYQDLPHLVNADGQYLFCRYWKPTGTPKALIFVSHG 60
*****
Ph-MGLL      AGEHCGRYEELACMLMGLDLLVFAHDHVGHGQSEGERMVVSDFHVFVRDVLQHVDSMQKD 120
Hs-MGLL      AGEHCGRYEELACMLMGLDLLVFAHDHVGHGQSEGERMVVSDFHVFVRDVLQHVDSMQKD 120
*****
Ph-MGLL      YPGLPVFLLGHSMGGAIAILTAAERPGHFAGMVLISPLVLANPESATTFKVLAAKVLNLV 180
Hs-MGLL      YPGLPVFLLGHSMGGAIAILTAAERPGHFAGMVLISPLVLANPESATTFKVLAAKVLNLV 180
*****
Ph-MGLL      LPNLSLGPIDSSVLSRNKTEVDIYNSDPLICRAGLKVCFGIQLLNAVSRVERALPKLTVP 240
Hs-MGLL      LPNLSLGPIDSSVLSRNKTEVDIYNSDPLICRAGLKVCFGIQLLNAVSRVERALPKLTVP 240
*****
Ph-MGLL      FLLLQGSADRLCDSKGAYLLMESAKSQDKTLKIYEGAYHVLHKELPEVTNSVFHEINMWV 300
Hs-MGLL      FLLLQGSADRLCDSKGAYLLMESAKSQDKTLKIYEGAYHVLHKELPEVTNSVFHEINMWV 300
*****
Ph-MGLL      SQRTATAGTASPP 313
Hs-MGLL      SQRTATAGTASPP 313
*****

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Figure 5. Comparison of predicted FAAH-ORF from the baboon (*Papio anubis*) and human (*Homo sapiens*)

The In bold: cytoplasmic domain; in italics: helical domain; underlined: extracellular motif.

Ph: *Papio spp*, Hs: *Homo sapiens*. The differences in amino acids composition between the two species are marked by rectangles.