A Short Communication on Sequential and Structural Information's of Human Galanin Receptors using in Silico Methods

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Abstract

Gal (1-3) receptors are members of GPCR superfamily with seven transmembrane helices. The neuropeptide galanin mediates its effects through the receptor subtypes Gal1, Gal2, and Gal3 and has been implicated in anxiety and depression related behaviors. Galanin receptors are considered to be important targets for the development of novel antidepressant drugs. Owing to the importance of these receptors, a short communication about the sequential and structural studies about the functional Galanin (1-3) receptors has been reported. Structural studies have been hampered due to the lack of X-ray crystal structures. However with the availability of templates with close homologs comparative modeling could be encouraging. Sequence analysis was done for each receptors and homology modeling of each receptors were done with recently reported templates. Comparative analyses were done between these receptors to identify the relationships between them sequentially. Phylogram was generated between these receptors to identify the close homologue between this receptor and found that Gal2 and Gal3 receptors are closer. Our results could be useful for further structure based drug design targeting Gal1, Gal2 and Gal3 receptors.

Key words : Gal1, Gal2, Gal3, Galanin, Homology Modeling

1. Introduction

Galanin is a widely distributed peptide neurotransmitter that activates G-protein coupled receptors to regulate a range of physiological processes, including feeding, insulin release, lactation, spinal reflex, gut contractility, growth, learning, memory, and depression^[1-4]. All 14 species homologs studied to date contain 29 amino acids and a carboxy-terminal amide, with the exception of human galanin which contains 30 residues (ending in Ser30) and a carboxyl free acid. All except tuna fish galanin^[5] share an absolutely conserved NH₂-terminal region associated with biologic activity (residues 1-14) and a variant COOH-terminal region^[2,6]. In the rat, galanin has been shown to have physiological effects in feeding, insulin release, lactation, gut contractility, and growth and has effects on central functions such as spinal reflex, learning, and memory and in rodent models of depression. Three cloned galanin GPCRs have been

identified and are designated as Gal1, Gal2, and Gal3^[7,8].

These Galanin receptors (1-3) are members of GPCR superfamily consist of seven transmembrane helices and they are among the preferred drug targets of the pharmaceutical industry. They have been implicated in anxiety and depression related behaviors and it has been mediated by the peptide termed as galanin neuropeptide. The pharmaceutical industry and academic researchers have been searching for non-peptide type galanin receptor ligands that would have better stability than galanin, the peptide that is metabolized within minutes in humans, and which would cross the blood-brain barrier to be able to act at the central galanin receptors.

In this short communication, Galanin receptors were selected for in silico studies. Till to date there is no X-ray structural report on the family of these receptors. There was only one report on the modeling of N-terminal region of galanin and its interaction with galanin receptor^[9]. However in that study the authors reported the interaction of N-gal with galanin subtype receptors. They obtained the Gal (1-3) receptors from ModBase database and analysis was done. With the availability of recently reported GPCR template structures, it could be interesting and useful to develop the homology models

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of Galanin receptors. This prompted us to initiate the analysis. This short study deals with the comparative modeling of Galanin receptors using recently reported template structures. Comparative analyses were done between these receptors to identify the relationships between them. We hope that our results could be helpful for future prospects.

2. Experimental Section

All molecular modeling calculations were performed using molecular modeling programs, Sybyl 8.1 and Modeller installed on a Linux environment.

2.1. Sequence Analysis of Galanin Receptors

The amino acid sequences of human Galanin receptors were retrieved from the Uniprot KB/TrEMBL database. The sequences of galanin receptors were further

used for template identification using the basic local alignment search tool for protein (BLAST) algorithm^[10,11] against the protein data bank^[12]. After the search, the alignment between the template and the target sequences was performed using ClustalW 2.0^[13].

2.2. Homology Modeling of Galanin Receptors

Homology modeling of each galanin receptor was performed using the Modeller9v4 program^[14-16]. Ten such 3D models were generated for these Galanin receptors with the alignment obtained using number of homologous template structures from the PDB for further computational study. Finally a model with the lower Molpdf (molecular probability density function) score and no significant main chain root mean square (RMS) deviation was selected. The selected models of Galanin receptors were refined by simple energy minimization and were further validated using PROCHECK^[17].

4EA3	-----PLGLKVTIVG-LYLAVCVGGLGNCLVMYVILR	32
4DJH	-----SPAIPVITA-VYSVVFVVLGVNSLVMFVILR	32
GALR1	MELAVGNLSEGNASWPEPPAPEPGPLFGIGVENFVTLVVFGILFALGVLSLVTVLAR	60
2Y00	-----AELLSQWEAGMSL-LMALVVLLIVAGNVLVIAIGS	36
	: : : : * * * : . :	
4EA3	HT--KMKTATNIYIFNLALADTLVLL-TLFFQGTDLILGFWPFGNALCKTVIAIDYYNMF	89
4DJH	YT--KMKTATNIYIFNLALADALVTT-TMFFQSTVYLMNSWPFGLVLCCKIVLSIDYYNMF	89
GALR1	SKPGKPRSTTNLFILNLSIADLAYLLFCIEFQATVYALPTWVLGAFICKFIHYFFTVSML	120
2Y00	TQ--RLQTLTNLFITSLACADLVVGLLVVFFGATLVVRGTWLGSLCELMWTSLDVLCVT	94
	: : * : * : * : * : * : * : * : :	
4EA3	TSTFTLTAMSVDRYVAICHP-----TSSKAQAVNVIAWALASVVGVPVAIMG-----	136
4DJH	TSIFTLTAMSVDRYIAVCHPVKALDFRTPKAKIINICIWLLSSSVGISAIVLG-----	143
GALR1	VSIFTLAAMSVDRYVAIVHSRRSSSLRVSRNALLGVGCIWALSAMASPVAYHQ-----	174
2Y00	ASITLCVIAIDRYLAITSFFRYQSLMTRARAKVITCTVWAIASALVSFLFIMMH-----	148
	. * * * : : * : * : . . . * : * : : . :	
4EA3	-----SAQVEDE--EIECLVEIP-TPQDYWGPFV-----AICIFLFSFIVEVL	176
4DJH	-----GTRVREDVDVIECSLQFPDDYSWWDLFM-----KICVFIFAFVIEVL	186
GALR1	-----GLFHPRASNQFFCWEQWPD---PRHKKAY-----VVCTFVFGYLLPL	214
2Y00	-----WWRDEDPQALKCYQD-----PGCCDFVT-----NRAYAIASSIISFYIPL	189
	* : : : : * : *	
4EA3	VISVCYSLMIRRLRGVRLLSG-SREKD-RNLRRITRLVLVVAVFVGCWTEPVQVFVLAQG	234
4DJH	IIIVCYTLMILRLKSVRLLSG--REKD-RNLRRITRLVLVVAVFVGCWTEPIHIFILVEA	243
GALR1	LICFCYAKVLNHLHKK-LKNM--SKKSEASKKTAQTVLVVVVVFVGISWLPHHIHLMAE	271
2Y00	IMIFVALRVYREAKEQ-----SRVMLMREHKALKTLGIIMGVFTLCWLEFFLVNIVNV	242
	: : . : . : . : : : : * * * * : . :	
4EA3	LGVPSSSETAVAILRFCTALGYVNSCLNPILYAFLDENFKACFR-----	278
4DJH	LGS-----AALSSYYFCIALGYTNSSLNPILYAFLDENFKRCFRDFCF-----	286
GALR1	FGVFPLTPASFIFRITAHCLAYSNSSVNPIIYAFLENFRKAYKQVFKCHIRKDSHLSDT	331
2Y00	FNRDL---VPDWLFVAFNWLGYANSAMNPITYC-RSPDFRKAFKRLA-----	286
	: . . . * * * : * : * : : : :	
4EA3	-----	
4DJH	--P-----	287
GALR1	KESKSRIDTPPSTNCTHV--	349
2Y00	-----	

Fig. 1. Sequence alignment between GAL1 and A chain of the top template structure hits.

3. Results and Discussion

3.1. Sequence Analysis of Galanin Receptors

In order to identify adequate template structures for each Galanin receptor modeling, Blast search was done. The search using Blast algorithm against the PDB revealed numerous potential templates for modeling. The Uniprot sequence of Gal1 (P47211) was retrieved from Uniprot database. Among the template hits, three top structures were selected as template hits. Top templates used for modeling was N/OFQ Opioid Receptor (PDB code: 4EA3)^[18], Beta1 Adrenergic Receptor (PDB code: 2Y00)^[19] and human kappa opioid receptor (PDB code: 4DJH)^[20]. The sequence identity between Galanin1 N/OFQ Opioid Receptor was found to be 34% with query coverage of 80%. Similarly, the identity between Gal1 and Beta1 Adrenergic Receptor was

found to be 29% with query coverage of 83%. The final template human kappa opioid receptor shared an identity of 39% and the query coverage was found to be 70%. The sequence conservation and the alignment were obtained using ClustalW and are shown in Fig. 1.

Similarly, the Uniprot sequence of Gal2 (O43603) was retrieved from Uniprot database. Top templates used for modeling was N/OFQ Opioid Receptor (PDB code: 4EA3)^[18], mu-opioid receptor (PDB code: 4DKL)^[21] and human kappa opioid receptor (PDB code: 4DJH)^[20]. The sequence identity between Gal2 and N/OFQ Opioid Receptor was found to be 35% with query coverage of 71%. Similarly, the identity between Gal2 and mu-opioid receptor was found to be 34% with query coverage of 73%. The final template human kappa opioid receptor shared an identity of 33% and the query coverage was found to be 71%. The sequence

4DKL	-----MVTAITIMALYSIVCVVGLFGNFLVMYVIVRYTKMKTAT	39
4DJH	-----SPAIPVIITAVYSVVFVVGVLGNSLVMFVIIRYTKMKTAT	40
4EA3	-----PLGLKVTIVGLYLAVCVGGLLGNCLVMYVILRHFKMKTAT	40
GALR2	MNVSGCPGAGNASQAGGGGWHPEAVIVPLLFALIFLVGTGNTLVLAVLLRGQAVSTT	60
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4DKL	NIYIFNLALADA-LATSTLPFQSVNYLMGTWPFGNILCKIVISIDYNNMFTSIFTLCTMS	98
4DJH	NIYIFNLALADA-LVTTTTPFQSTVYLMNSWPFQDVLCIKIVLSIDYNNMFTSIFTLTMS	99
4EA3	NIYIFNLALADT-LVLLTLFPQSTDLLGFWPFGNALCKTVIAIDYNNMFTSTFTLTMS	99
GALR2	NLFILNLGVADLCFILCCVFPQATITLDGWVFGSLCKAVHFLIFLTMHASSFTLAAYS	120
	* : : * : * : * : : * : * : * : * : * : * : * : * : * : *	
4DKL	VDRYIAVCHPVKALDFRTPRNAKIVNVNWLSSAIGLPMFMATKYRQ--GSIDCTLT	156
4DJH	VDRYIAVCHPVKALDFRTPKAKIINICIWLLSSSVGISAIVLGGTKVREDVDVIECSLQ	159
4EA3	VDRYVAICHPE-----TSSKAQAVNVAIWALASVVGVEVVAIMGSAQVED--EEIECLVE	150
GALR2	LDRYLAIKRYPLHSRELRTFERNALAAIGLIWGLSLFSGPYLSYYRQSLA--NLTVCHPA	178
	: * : * : * : * : * : * : * : * : * : * : * : * : * : *	
4DKL	FS-HPTWYWENLLKICVFIFAFIMPVLIITVCYGLMILRL-KSVRE-----KDRNLR	206
4DJH	FPDDDYSWWDLFMKICVFIFAFVIVPVLIIIVCYTLMILRL-KSVR-LLSG-RE-KDRNLR	215
4EA3	IP-TPQDYWGPVFAICIFLFSFIVPVLVISVCYSLMIRRL-RGVR-LLSG-SREKDRNLR	206
GALR2	WS---APRRRAMDICTFVFSYLLPVLVLGLTYARTLRYLWRAVD-PVAA-GSGARRAKR	232
	: * * * : * : : * : * : * : * : * : * : * : * : * : *	
4DKL	RITRMVLVVAVFIVCWTPPIHIYVIAKALITIPETTFQTVSWHFCIALGYTNSCLNPVLY	266
4DJH	RITRLVLVVAVFVVCWTPPIHIFILVEALGS-----AALSSYYFCIALGYTNSCLNPILY	270
4EA3	RITRLVLVVAVFVVCWTPVQVFLAQLGQVPSSETAVAILRFTALGYVNSCLNPILY	266
GALR2	KVTRMILIVAALFCLCWMPEHAILCVWFGQFPLTRATYALRLSHLVSYANSVNPVIVY	292
	: * : * : * : * : * : * : * : * : * : * : * : * : * : *	
4DKL	AFLDENFKRCF-----R-----EF-----	280
4DJH	AFLDENFKRCF-----R-----DF-----	284
4EA3	AFLDENFKACF-----R-----	278
GALR2	ALVSKHFRKGFRTICAGLLGRAPGRASGRVCAAAARGTHSGSVLERESSDLLHMSBAAGAL	352
	* : : : * : * : *	
4DKL	-----CI-----	282
4DJH	-----CF-----P-----	287
4EA3	-----	
GALR2	RPCPGASQPCILEPCPGPSWQGPAGDSILTVDA--	387

Fig. 2. Sequence alignment between GAL2 and A chain of the top template structure hits.

4DKL	-----MVTAITIMALYSIVCVVGLFGNFLVMYVIVR-----YTKMKTATNIY	42
4DJH	-----SPAIPVIITAVYSVVVGLVGNLSLVMFVIIR-----YTKMKTATNIY	43
4EA3	-----PLGLKVTIVGLYLAVCVGGLGNCLVMYVILR-----HTKMKTATNIY	43
GALR3	MADAQNISLDSPGSVGAVAVPVFALIFLLGTVGNGLVLAVLLQFPGPSAWQEPGSGTTDLF	60
	: : : : : * . ** ** : : : : : : : : : :	
4DKL	IFNLALADA-LATSTLEFFQSVNYLMGTWFFGNILCKIVISIDYYNMFTSIFTLTCTMSVDR	101
4DJH	IFNLALADA-LVTTTMEFFQSTVYLMNSWFFGDVLCKIVLSIDYYNMFTSIFTLTCTMSVDR	102
4EA3	IFNLALADT-LVLLTLEFFQGTDLGLGFWFFGNALCKTVIAIDYYNMFTSTFTLTCTMSVDR	102
GALR3	ILNLAVADLCFILCCVFFQATITVLDANLFGALVCKAVHLLIYLTMYASSFTLAASVDR	120
	* : ** : : : : * : : : : * : : : : * : : : : *	
4DKL	YIACHPVKALDFRTPRPAKIVNVNWLSSAIGLPVFMATTKYRQ--GSIDCTLTFS-	158
4DJH	YIACHPVKALDFRTPKAKIINICIWLLSSSVGISAIVLGGTKVREDVDIECSLQFPD	162
4EA3	YVAICHP-----TSSKAQAVNVVAIWALASVVGVPVAIMGSAQVED--EEIECLVEIP-	152
GALR3	YLAVRHPLRSRALRTPRPAKIAAVGLVWLLAALFSAPYLSYGTVRYG--ALELCVPAW--	176
	* : : * : : * : : : : * : : : : *	
4DKL	HPTWYWNELKICVFIFAFIMPVLIIITVCYGLMILRLKSVRE-----KDRNLRR	207
4DJH	DDYSWMDLFMKICVFIFAFVIVPLIIIVCYTLMILRLKSVR-LLSGR----E-KDRNLRR	216
4EA3	TPQDYWGVPFAICIFLFSFIVPVLVISVCYSLMIRRLRGVR-LLSG-S---REKDRNLRR	207
GALR3	--EDARRRALDVATFAAGYLLPVAVVSLAYGRITLRFLLWAAV-GEAG-AAAAEARRRATGR	232
	: : . * : : : * : : : : * : : : : *	
4DKL	ITRMVLVVAVFIVCWTFPIHIYVVIKALITIPETTFQTVSWHFCIALGYTNSCLNPLVYA	267
4DJH	ITRLVLVVAVFVVCWTFPIHIFILVEALGS-----AALSSYFPCIALGYTNSCLNPLIYA	271
4EA3	ITRLVLVVAVFVGCWTFVQVFLAQLGLVQPSSETAVAILRFTALGYVNSCLNPLIYA	267
GALR3	AGRAMLAVALYALCWGPHHALILCFWYGRFAFSPATYACRLASHCLAYANSCLNPLVYA	292
	* : * . * : : * : : : : : : : : : * : * . * . * : : *	
4DKL	FLDENFKRCFR-----EF-----C-----I	282
4DJH	FLDENFKRCFR-----DF-----C-----F	286
4EA3	FLDENFKACFR-----	278
GALR3	LASRHFRFRRLWPCGRRRRHRRARRRVRPSSGPPGCPGDARPSGRLLAGGGQGPE	352
	: . . : * : *	
4DKL	-----	
4DJH	P-----	287
4EA3	-----	
GALR3	PREGPVHGGGAARGPE--	368

Fig. 3. Sequence alignment between GAL3 and A chain of the top template structure hits.

conservation and the alignment were obtained using ClustalW and are shown in Fig. 2.

Likewise, the Uniprot sequence of Gal3 (O60755) was retrieved from Uniprot database. Top templates used for modeling was N/OFQ Opioid Receptor (PDB code: 4EA3)^[18], mu-opioid receptor (PDB code: 4DKL)^[21] and kappa opioid receptor (PDB code: 4DJH)^[20]. The sequence identity between Gal3 and N/OFQ Opioid Receptor was found to be 33% with query coverage of 74%. Similarly, the identity between Gal3 and mu-opioid receptor was found to be 31% with query coverage of 76%. The final template human kappa opioid receptor shared an identity of 31% and the query coverage was found to be 76%. The sequence conservation and the alignment were obtained using ClustalW and are shown in Fig. 3.

3.2. Homology Modeling of Galanin Receptors

The coordinates of the crystal structures of respective template hits were used to build the homology model of Galanin receptors (Gal 1-3). In these models, the seven transmembrane (TM) helices were properly transformed according to that of the template structures. The three dimensional homology models of Galanin receptors were generated using Modeller9v4 program. Ten models were developed for each receptor and finally the model with the lowest MolPdf as well as with the lowest RMSD was selected and it is shown in Fig. 4. The selected models were further validated stereochemically using additional parameters such as the PROCHECK plots. The statistical parameters obtained for the Galanin models are summarized in Table 1.

Fig. 4. Homology models of Galanin receptor structures. Seven transmembrane helices are colored in different color codings. N-Terminal and C-Terminal loop regions are deleted for clarity

3.3. Sequence Analysis of Human Functional Galanin Receptors

Sequence analysis between these Galanin receptors was done using ClustalW and the relationship between these receptors was analyzed sequentially. Phylogram

Table 1. Validation results of Galanin receptor homology models

Model	Procheck			
	Core %	Generously allowed %	Additionally allowed %	Disallowed %
Gal1	93.5	5.2	0.3	1.0
Gal2	92.4	4.6	2.1	0.9
Gal3	90.9	5.5	1.9	1.6

was generated to analyze the phylogenetic relationship between these receptors. Our results found that Gal2 and Gal3 receptors shared more identity than others. It was identified that Gal1 and Gal2 shared an identity of 36 %, whereas Gal1 and Gal3 shared the sequence identity of 33%. It was found that Gal2 and Gal3 shared an identity of 53%. Overall, our sequence analysis found that Gal2 and Gal3 receptor are found to be close. Phylogram results confirmed that both Gal2 and Gal3 are closer than Gal1. The alignment obtained between these

sp O43603 GALR2	-MNVSGCPGAGNAGAGGGG-----WHFEAVIVPELFLALIFLVGTUGNVLVLAFLR	52
sp O60755 GALR3	-----MADAQNISLSDSPGS-----VGAVAVPVVFLALIFLVGTUGNVLVLAFLR	44
sp P47211 GALR1	MELAVGNLSEGNASWPEPPAPEGLPGIGVENFVTLVVFGLIFALGVGNLSVITVLAR	60
	. * * . . . : * : * : * : * : * : * : *	
sp O43603 GALR2	GG-----QAVSTTNLFILNLGVADLCFILCCVFOATITVLDGWFVGSLLCKAVHFLIFL	107
sp O60755 GALR3	PGPSAMQEPGSTTDLFILNLAVADLCFILCCVFOATITVLDGWFVGSLLCKAVHFLIFL	104
sp P47211 GALR1	SKPG--KPRSTTNLFILNLGIADLAILFCIFFOATVYALPTWVLGAFICKFIHYFTV	117
	: . * : * : * : * : * : * : * : * : * : *	
sp O43603 GALR2	TMHASSFTLAAVSLDRYLAIYPLHSRELRTPRNALAAIGLWGLSLLSGPYLSYVR--	165
sp O60755 GALR3	THYASSFTLAAVSLDRYLAIYPLHSRELRTPRNALAAIGLWGLSLLSGPYLSYVR--	162
sp P47211 GALR1	SMLVSIPTLAASVDYVAIVHSRRSSSLRVSRNALLGVGCIWALSIAASPV-VAYHQGL	176
	: * . * * : * : * : * : * : * . * : * : * : * : *	
sp O43603 GALR2	-QSQLANLTVCHFAWSAPR--RRAMDICTVPSYLLPVVLGLTYARTLYLWRAVDPV--	221
sp O60755 GALR3	-TVRYGALELCVEAWEDAR--RRALDVATFAAGYLLPVVAVSLAYGRTRFLWAAVGPAGA	220
sp P47211 GALR1	FHFRASNQTFCWEQWDPDRHKAYVUCTFVFGYLLPLLLICFCYAKVLNHLHKKLNMS-	235
	: . . * * . * : * : * : * : * : * : * : * : *	
sp O43603 GALR2	AAGSGARRAKRRVTRMILVAALFCLCWPHHALILCVWFQFPLTRATYALRILSHLVS	281
sp O60755 GALR3	AAAEARRRATGRAGRAMLAVALYALCWGPHHALILCFWYGRFAPSPATYACRLASHCLA	280
sp P47211 GALR1	---KKSEASKKTAQTVLVVVVVFGISWLPHHIHLMAEFVGFPLTPASFLFRITAHCLA	292
	. . . : . : * : * : * : * : * : * : * : * : * : *	
sp O43603 GALR2	YANSCVNPIYALVSKHFRKGFRTICAGLLGRAPGRASGRVCAAAARGTHSGSVLERESSD	341
sp O60755 GALR3	YANSCVNPIYALVSKHFRKGFRTICAGLLGRAPGRASGRVCAAAARGTHSGSVLERESSD	341
sp P47211 GALR1	YANSCVNPIYALVSKHFRKGFRTICAGLLGRAPGRASGRVCAAAARGTHSGSVLERESSD	341
	* : * : * : * : * : * : * : * : * : * : * : * : *	
sp O43603 GALR2	LLHMSBAAGALRCPGASQPCILECPGPGSWQGPAGDSILTVDVA	387
sp O60755 GALR3	CPGDARPSGRLLAGGQGE-----EPREGVHGGGAARGPE-----	368
sp P47211 GALR1	CTHV-----	349

Fig. 5. Sequence alignment between Galanin receptors and the phylogenetic evolution between these receptors.

receptors and the phylogram developed between these receptors with their distances is shown in Fig. 5.

4. Conclusion

In this short communication, sequence analysis was done for each galanin receptor to identify the template structures for modeling. Homology modeling was then performed to develop the homology models of Galanin receptors using the more recently reported template structures. Comparative analyses were done between these receptors to identify the relationships between them. We hope that our results could be useful for further structure based drug design targeting Gal1, Gal2, and Gal3 receptors.

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