Molecular evolution of receptors for eicosanoids

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Abstract The amino acid sequences of the receptors for various prostaglandins, thromboxane and lipoxin, which belong to the rhodopsin family, were aligned, and a phylogenetic tree was constructed to infer the evolutionary history of the arachidonic acid cascade. The obtained tree suggested that the origin of the cyclooxygenase pathway was different from that of the lipoxygenase pathway. The receptors involved in the cyclooxygenase pathway constructed an independent cluster, but the lipoxin A_4 receptor, which is involved in the lipoxygenase pathway, belonged to the cluster of peptide receptors. The primitive form of the cyclooxygenase pathway had been a signal transductiuon system composed of prostaglandin E_2 and its receptor associated with cAMP metabolism.

Key words: Eicosanoids receptor; Rhodopsin family; Phylogenetic tree

1. Introduction

A wide variety of bioactive compounds, which include PGs, TXs, leukotrienes and LXs are synthesized from arachidonic acid. The bioactive compounds are collectively termed eicosanoids and the metabolic pathway to produce such compounds is called the arachidonic acid cascade (for reviews see [1–3]). The arachidonic acid cascade is divided into four pathways, i.e. the cyclooxygenase pathway, lipoxygenase pathway, cytochrome *P*-450 pathway and autooxygenation pathway. PGs and TXs are synthesized in the cyclooxygenase pathway. On the other hand, leukotrienes and LXs are synthesized in the lipoxygenase pathway. The physiological actions of the eicosanoids are mediated through the corresponding receptors.

Approaches with recombinant DNA techniques have revealed various aspects of the receptors for eicosanoids at a molecular level. The cDNAs and/or genes of TXA receptors from human [4,5], mouse [6] and rat [7] have been cloned and sequenced. The nucleotide sequences of cDNAs of receptors for PGs have also been determined, and assigned to four groups, PGD receptor[8], PGE receptor [9–19], PGF receptor [20–22], and PGI receptor [23–25]. The PGE receptors have been pharmacologically classified into four subtypes, EP_1, EP_2, EP_3 and EP_4, based on their agonist/antagonist specificity and signal transduction [26]. EP_1 couples to PI turnover and calcium mobilization, EP_2 and EP_4 to stimulation of adenylate cyclase and EP_3 to inhibition of adenylate cyclase. Four groups of PGE receptors encoded by different genes have been cloned. Among them, the receptor originally cloned by Watabe et al. [10] and that by Sugimoto et al. [12] correspond to the pharmacologically-defined EP_1 and EP_3 receptors, respectively. The other two species of cloned PGE receptors couple positively to adenylate cyclase and have been reported to be EP_2 subtype. They are the one cloned originally by Honda et al. [9] and another recently cloned by Regan et al. [19]. While both receptors are sensitive to some of the EP_2 agonists such as 11-deoxy PGE_1, the former is insensitive to butaprost, a typical EP_2 agonist. In addition, a recent study in our laboratories has revealed that it is sensitive to an EP_4 antagonist, AH23848, indicating that it is EP_4 and not EP_2**. We, therefore, call the 'EP_2' receptor originally cloned by Honda et al. [9] EP_4, and that by Regan et al. [10] EP_2, in the present study. The EP_3 subtype has multiple isoforms generated by alternative splicing [13,15,16,27]. On the other hand, the receptor for LXA, which is involved in the lipoxygenase pathway, has been reported to be related to the formyl peptide receptor [28]. The receptors for PGs, TXA and LXA show similarity in amino acid sequence to the members of the rhodopsin family. The rhodopsin family includes not only the photoreceptors or rhodopsins but also the receptors for peptide hormones, neurotransmitters and odorant substances. The members of the family have seven membrane-spanning regions in their amino acid sequences and are coupled with the corresponding G-proteins for signal transduction, which is also the case with the receptors for eicosanoids.

Computer-assisted sequence comparison is a popular and practical method to investigate molecular evolution. As described above, the sequence data of the receptors involved in the cyclooxygenase pathway and lipoxygenase pathway have been accumulated, which are useful to infer not only the evolutionary relationships among the receptors but also the evolutionary history of the arachidonic acid cascade. For the investigation of the evolutionary aspects, the amino acid sequences of the receptors for PGs, TXA and LXA were aligned and a phylogenetic tree was constructed.

2. Materials and methods

2.1. Multiple alignment

Twenty receptors for various eicosanoids, together with ten peptide receptors and two PAF receptors, were aligned by a computer program with the tree-based algorithm [29]. The McLachlan's score matrix [30]

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Abbreviations: TX, thromboxane; PG, prostaglandin; LX, lipoxin; TXA, thromboxane A_2; PGD, prostaglandin D_2; PGE, prostaglandin E_2; PGF, prostaglandin $F_{2\alpha}$; PGI, prostacyclin; LXA, lipoxin A_4; PAF, platelet-activating factor.

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was used for the alignment. Highly diverged regions were found in the N- and C-terminal regions and the extra- and intracellular domains of the receptors, where alignment was neither reliable nor suitable for phylogenetic analysis. Therefore, the diverged regions were excluded from the alignment. The excluded C-terminal regions of EP_3 subtypes contained the amino acid sequences generated by alternative splicing. The remaining regions were modified by visual inspection to increase similarity.

2.2. Construction of phylogenetic tree

The genetic distance for every pair of the aligned sequences was calculated as the sequence difference with Poisson correction [31]. The sites containing gaps were excluded from the calculation. Then, a phylogenetic tree was constructed by the neighbor-joining method [32] using the genetic distances. Statistical significance for obtained tree topology was evaluated by bootstrap analysis [33,34] with one thousand iterations of bootstrap resampling and tree reconstruction.

3. Results and discussion

The amino acid sequences of the nineteen receptors for PGs and TXA were available. The reports on the sequences told that the receptors show higher sequence similarity to one another than to the other members of the rhodopsin family, which suggests that they construct a cluster in the phylogenetic tree of rhodopsin family. Contarary to that, the LXA receptor, which is related to the formyl peptide receptor [28], showed close resemblance to the other peptide receptors rather than to the receptors for PGs and TXA. PAF is not an eicosanoid, but it is another kind of lipid-derived mediator. PAF receptor also belongs to the rhodopsin family [35–38]. To investigate the evolutionary relationships within the receptors, the amino acid sequences of the receptors including ten peptide receptors were aligned (see Fig. 1). A phylogenetic tree of the receptors was constructed according to the alignment.

The tree was divided into two clusters. One of them consisted of the receptors for PGs and TXA. Another cluster consisted of peptide receptors, which also included PAF receptors and LXA receptor. The root of the tree was putatively introduced to the midpoint of the branch connecting the two clusters. The root node of each cluster ((a) and (b) in Fig. 2) was identified using another cluster as an outgroup. Regan et al. [19] previously investigated the phylogenetic tree of the PG and TXA receptors derived only from human. The topology of the former cluster in our tree was consistent with that of Regan et al., although the receptors of PGD and PGI were not included in their tree, both of which were present in this cluster. Furthermore, we included in our analysis the LXA receptor and receptors for PGs and TXA from mammals other than human to infer evolution of the receptors in the lipoxygenase pathway and to discuss the node for mammalian divergence.

The root point of the former cluster was identified as node (a) in Fig. 2, where the cluster of the receptors was divided into two subclusters. One of them included the receptors for PGI, PGD and PGE (EP_2 and $_4$ subtypes). The subcluster is here referred to as CL-1. On the other hand, another subcluster was further divided into two subclusters, CL-2 and CL-3. It is known that the signal transduction through the receptors in the CL-1 is associated with an increase in intracellular cAMP. CL-1 is further divided into two subclsuters. One of them consisted of EP_4, and another of EP_2, PGD and PGI receptors. CL-2 consisted of the receptors for TXA, PGF and PGE (EP_1 subtype), which mediate the signal transduction through an increase in inositol triphosphate. CL-3 was composed of the PGE receptors (EP_3 subtype). The signal transduction through EP_3 subtype is associated with a decrease in cAMP, although an isoform of EP_3 subtype generated by alternative splicing is coupled to both stimulation and inhibiton of adenylate cyclase and stimulaton of PI turnover [13,27].

All the subclusters included PGE receptors although the subtypes in the subclusters were different from each other. The evolutionary position of PGE receptors in the tree suggested that the cyclooxygenase pathway may have been initiated as a system composed of PGE and its receptor. Furthermore, it is known that the activation of the members of CL-1 and CL-3 are associated with the cAMP metabolisms. So, the primitive PGE receptor may have mediated signal transduction through cAMP metabolisms as Regan et al. recently suggested in their paper [19]. The node (a) was considered to correspond with a gene duplication which may have elaborated the primitive PGE receptor to form two functionally different receptors, that is, a PGE receptor associated with an increase of cAMP and that with a decrease of cAMP. The former was subjected to further gene duplication to generate EP_2 and EP_4 subtypes. Then, PGI and PGD receptors diverged from the EP_2 subtype. The tree suggested that both the functional divergence between EP_4 and EP_2 and that between PGI and PGD receptors occurred before mammalian divergence. The latter was an ancestral receptor for EP_3 subtype, from which ancestral receptor for EP_1 subtype had diverged by gene duplication at the node (c). The ancestral EP_1 subtype had been able to mediate signal transduction through inositol triphosphate instead of cAMP. The ancestral EP_1 receptor had further diverged by gene duplications to form the receptors for PGF and TXA. On the other hand, the ancestral EP_3 subtype has acquired the mechanism of alternative splicing to show several functions without gene duplication. Considering that the EP_3 subtypes from mouse, rat and rabbit have multiple isoforms, the subtype may have been subjected to alternative splicing before mammalian divergence. In addition, the tree suggested that all the variety of the receptors for PGs and TXA have been present before mammalian divergence, that is, cyclooxygenase pathway may have been established before mammalian divergence.

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Fig. 1. A multiple alignment of receptors for PGs, TXA, LXA, PAF and peptides. The integer in parentheses of each sequence indicates the position of the neighboring residue in the primary structure. The abbreviated name of each sequence is shown at the left side to the alignment. MPGI and HPGI; mouse [25] and human [23] PGI reeptors, MPGD; mouse PGD receptor [8], HEP2; human PGE receptor (EP_2 subtype) [19], HEP4, MEP4, and REP4, human [17], mouse [9] and rat [18] PGE receptors (EP_4 subtype), MTXA, RTXA and HTXA; mouse [6], rat [7], human [4] TXA receptors, BPGF, MPGF, RPGF; bovine [21], mouse [20] and rat [22] PGF receptors, HEP1 and MEP1; human [11] and mouse [10] PGE receptors (EP_1 subtype), HEP3, RBEP3, MEP3 and RTEP3; human [14], rabbit [16], mouse [12] and rat [15] PGE receptors (EP_3 subtype), HSBP; human substance P receptor [39], HNMK; human neuromedin K receptor [40], HNPY; human neuropeptide Y receptor [41], HGRP; human gastrin-releasing peptide receptor [42], HNMB; human neuromedin B receptor [42], HET1; human endothelin-1 receptor [43], GPAF and HPAF; guinea-pig [35] and human [36] PAF receptors, HC5A; human C5A anaphylatoxin chemotactic receptor [44], HIL8; human high affinity interleukin-8 receptor A [45], HLPX; human LXA receptor [46], HBRB2;human B2 bradykinin receptor [47], HAG2; human type-1 angiotensin-II receptor [48]. '–' indicates gap.

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MPGI   ( 50) STL-MFVA-GVVGNGLALGILG   ( 81) VLVTGLAVTDLLGTCFLSPA-VFVAYA   (121) LCDTFAFAMTFFGLASTLILFAMAVERCLALSHPYLY
HPGI   ( 20) STL-MFVA-GVVGNGLALGILS   ( 51) VLVTGLAATDLLGTSFLSPA-VFVAYA   ( 91) LCDAFAFAMTFFGLASMLILFAMAVERCLALSHPYLY
MPGD   ( 21) MGAVLFGA-GLLGNLLALVLLA   ( 62) VLVCGLTVTDLLGKCLISPM-VLAAYA   (103) LCETFAFLMSFFGLASTLQLLAMAVECWLSLGHPFFY
HEP2   ( 27) ISSVMFSA-GVLGNLIALALLA   ( 69) VLVTELVFTDLLGTCLISPV-VLASYA   (108) ACTYFAFAMTFFSLATMLMLFAMALERYLSIGAPLLL
HEP4   ( 23) IPAVMFIF-GVVGNLVAIVVLC   ( 56) TLVCGLAVTDLLGTLLVSPV-TIATYM   ( 91) LCEYSTFILLFFSLSGLSIICAMSVERYLAINHAYFY
MEP4   ( 48) IPAVMFIF-GVVGNLVAIVVLC   ( 81) TLVCGLAVTDLLGTLLVSPV-TIATYM   (116) LCDYSTFILLFFGLSGLSIICAMSIERYLAINHAYFY
REP4   ( 23) IPAVMFIF-GVVGNLVAIVVLC   ( 56) TLVCGLAVTDLLGTLLVSPV-TIATYM   ( 91) LCDYSTFILLFFGLSGLSIICAMSIERYLAINHAYFY
MTXA   ( 30) FAA-SFCALGLGSNLLALSVLA   ( 64) ALLCGLVLTDFLGLLVTGAI-VASQHA   (103) LCYFMGVAMVFFGLCPLLLGAAMASERFVGITRPFSR
RTXA   ( 30) FAA-SFCALGLGSNLLALSVLA   ( 64) ALLCGLVLTDFLGLLVTGAV-VASQHA   (103) LCHFMGAAMVFFGLCPLLLGAAMAAERFVGITRPFSR
HTXA   ( 30) FAA-SFCVVGLASNLLALSVLA   ( 65) TFLCGLVLTDFLGLLVTGTI-VVSQHA   (104) LCRFMGVVMIFFGLSPLLLGAAMASERYLGITRPFSR
BPGF   ( 32) FSI-IFMTVGILSNSLAIAILM   ( 68) LLASALVITDFFGHLINGTIAVFV-YA   (107) LCSIFGICMVFSGLCPLFLGSLMAIERCIGVTKPIFH
MPGF   ( 32) FSI-IFMTVGILSNSLAIAILM   ( 68) LLASGLVITDFFGHLINGGIAVFV-YA   (107) LCSIFGISMVFSGLCPLFLGSAMAIERCIGVTNPIFH
RPGF   ( 32) FSI-IFMTVGIVSNSLAIAILM   ( 68) LLASGLVITDFFGHLINGGIAVFV-YA   (107) LCSVFGISMVFSGLCPLFLGSTMAIERCIGVTNPLFH
HEP1   ( 38) LPI-FSMTLGAVSNLLALALLA   ( 75) LFVASLLATDLAGHVIPGAL-VLRLYT   (109) ACHFLGGCMVFFGLCPLLLGCGMAVERCVGVTRPLLH
MEP1   ( 40) LPI-FSMTLGAVSNVLALALLA   ( 77) LFVASLLAIDLAGHVIPGAL-VLRLYT   (111) ACHFLGGCMVFFGLCPLLLGCGMAVERCVGVTQPLIH
HEP3   ( 31) FPITMLLT-GFVGNALAM-LLV   ( 67) LCIGWLALTDLVGQLLTPV-VIVVYL   (106) LCTFFGLTMTVFGLSSLFIASAMAVERALAIRAPHWY
RBEP3  ( 50) FPITMLIT-GFVGNALAM-LLV   ( 86) LCIGWLALTDLVGQLLTSPV-VILVYL   (125) LCTFFGLTMTVFGLSSLFIASAMAVERALAIRAPHWY
MEP3   ( 31) FPITMMVT-GFVGNALAM-LLV   ( 67) LCIGWLALTDLVGQLLTSPV-VILVYL   (106) LCTFFGLTMTVFGLSSLLVASAMAVERALAIRAPHWY
RTEP3  ( 31) FPITMMVT-GFVGNALAM-LLV   ( 67) LCIGWLALTDLVGQLLTSPV-VILVYL   (106) LCTFFGLTMTVFGLSSLLVASAMAVERALAIRAPHWY
HSBP   ( 39) YTV-IVV-TSVVGNVVVMWIIL   ( 69) YFLVNLAFAEASMAAF-NTVVNFT-YA   (104) YCKFHNFFPIAAVFASIYSMTAVAFDRYMAIIHPLQP
HNMK   ( 91) YGV-VVA-VAVLGNLIVIWIIL   (121) YFLVNLAFSDASMAAF-NTLVNFI-YA   (156) YCRFQNFFPITAVFASIYSMTAIAVDRYMAIIDPLKP
HNPY   ( 47) YGA-VII-LGVSGN-LALIIII   ( 77) ILIVNLSFSDLLVAIMCLPF-TFV-YT   (112) MCKLNPFVQCVSITVSIFSLVLIAVERHQLIINPRGW
HGRP   ( 47) YGV-IIL-IGLIGNITLIKIFC   ( 77) LFISSLALGDLLLLITCAPV-DAS-RY   (112) GCKLIPFIQLTSVGVSVFTLTALSADRYKAIVRPMDI
HNMB   ( 50) YLL-IIT-VGLLGNIMLVKIFI   ( 80) IFISNLAAGDLLLLLTCVPVDASR-YF   (115) GCKLIPVIQLTSVGVSVFTLTALSADRYRAIVNPMDM
HET1   ( 86) ISCTIFI-VGMVGNATLLRIIY   (117) ALIASLALGDLIYVVIDLPI-NVF-KL   (157) LCKLFPFLQKSSVGITVLNLCALSVDRYRAVASWSRV
GPAF   ( 22) YSI-IFV-LGIIANGYVLWVFA   ( 54) IFMVNLTVADLLFLIT-LPLWIVY-YS   ( 89) LCNLAGCLFFINTYCSVAFLGVITYNRFQAVKYPIKT
HPAF   ( 22) YSI-IFV-LGVIANGYVLWVFA   ( 54) IFMVNLTMADMLFLIT-LPLWIVY-YQ   ( 89) LCNVAGCLFFINTYCSVAFLGVITYNRFQAVTRPIKT
HC5A   ( 44) FAV-VFL-VGVLGNALVVWVTA   ( 73) IWFLNLAVADFLSCLA-LPILFTS-IV   (108) ACSILPSLILLNMYASILLLATISADRFLLVFKPIWC
HIL8   ( 46) YAL-VFL-LSLLGNSLVMLVIL   ( 76) VYLINLALADLLFALT-LPIWAAS-KV   (109) LCKVVSLLKEVNFYSGILLLACISVDRYLAIVHATRT
HLPX   ( 33) LGV-TFV-LGVLGNGLVIWVAG   ( 62) ICYLNLALADFSFTAT-LPFLIVS-MA   ( 97) LCKLIHIVVDINLFGSVFLIGFIALDRCICVLHPVWA
HBRB2  ( 36) FLWVLFV-LATLENIFVLSVFC   ( 67) IYLGNLAAADLILACG-LPFWAIT-IS   (102) LCRVVNAIISMNLYSSICFLMLVSIDRYLALVKTM-S
HAG2   ( 35) YSI-IFV-VGIFGNSLVVIVIY   ( 65) VFLLNLALADLCFLLT-LPLWAVY-TA   (100) LCKIASASVSFNLYASVFLLTCLSIDRYLAIVHPMKS

MPGI   (168) FALPSIYAFCCLFCSLPLLGLGEH   (198) SWC-FIRM-R   (212) CAFSLAYASLM-ALLVTSIFFCNGSVTL-SL
HPGI   (138) LALPAIYAFCVLFCALPLLGLGQH   (168) SWC-FLRM-R   (182) AAFSLAYAGLV-ALLVAAIFLCNGSVTL-SL
MPGD   (150) LVAPVVAAFCLAFCALPFAGFGKF   (180) TWC-FIQMIH   (196) IGFSVLYSSLM-ALLVLATVVCNLGAMY-NL
HEP2   (155) GVLPVIYAVSLLFCSLPLLDYGQY   (185) TWC-FIRHGR   (194) TAYLQLYATLL-LLLIVSVLACNFSVIL-NL
HEP4   (138) LTLFAVYASNVLFCALPNMGLGSS   (168) TWC-FIDWTT   (182) AAYSYMYAGFS-SFLILATVLCN-VLVCGAL
MEP4   (163) LTLFAIYASNVLFCALPNMGLGRS   (193) TWC-FIDWTT   (207) AAFSYMYAGFS-SFLILATVLCN-VLVCGAL
REP4   (138) LTLFAVYASNVLFCALPNMGLGRS   (168) TWC-FIDWTT   (182) AAFSYMYAGFS-SFLILATVLCN-VLVCGAL
MTXA   (149) ATVGLVWVAAGALG-LPLLGLGRY   (179) SWC-FLTLGT   (192) VVFGLIFALLG-SASVGLSLLLNTVSVA-TL
RTXA   (149) ATVGLVWVGAGTLGLLPLLGLGRY   (179) SWC-FLTLGA   (192) VAFGLMFALLG-SVSVGLSLLLNTVSVA-TL
HTXA   (151) ATVGLVWAAALALGLLPLLGVGRY   (181) SWC-FLTLGA   (194) VAFGLLFSMLG-GLSVGLSFLLNTVSVA-TL
BPGF   (154) MMLSGVCFFAVFVALLPILGHRDY   (184) TWC-FYKTDE   (199) RFYLLLFAFLG-LLALGISFVCNAITGI-SL
MPGF   (154) MILSGVCMFAVFVAVLPILGHRDY   (184) TWC-FYNTEH   (199) RFYLLFFSFLG-LLALGVSFSCNAVTGV-TL
RPGF   (154) MILSGVCMFAVFVALLPILGHRDY   (184) TWC-FYNTEH   (199) RFYLLFFSSLG-LLALGISFSCNAVTGV-TL
HEP1   (156) LALAAVAAVALAVALLPLARVGRY   (186) TWC-FIGLGP   (201) ALLAGLFASLG-LVALLAALVCNTLSGL-AL
MEP1   (158) LALAVLAAMALAVALLPLVHVGRY   (188) TWC-FISLGP   (203) ALLAGLFAGLG-LAALLAALVCNTLSGL-AL
HEP3   (153) AVLLGVWLAVLAFALLPVLGVGQY   (183) RWC-FISTGR   (205) LFFASAFAFLG-LLALTVTFSCN-LATIKAL
RBEP3  (172) AVLLGVWLAVLAFALLPVLGVGQY   (202) TWC-FISTGR   (224) LFFASTFAFLG-LLALAITFACN-LATIKAL
MEP3   (152) PVLLGVWLSVLAFALLPVLGVGRY   (182) TWC-FISTGP   (204) VAFASAFACLG-LLALVVTFACN-LATIKAL
RTEP3  (152) PVLLGVWLSVLAFALLPVLGVGRY   (182) TWC-FISTGP   (204) VAFASAFACLG-LLALVVTFACN-LATIKAL
HSBP   (149) VVICVIWVLALL-LAFPQGYYSTT   (178) VVCMIE-WPE   (194) KVYHICVTVLIYFLPLLVIGYAYTVVGI-TL
HNMK   (201) IVIGSIWILAFL-LAFPQCLYSKT   (230) TLC-FVQWPE   (244) FTYHIIVIILVYCFPLLIMGITYTIVGI-TL
HNPY   (157) VGIAVIWVLAVA-SSLPFLIYQVM   (196) YVC-FDQFPS   (209) LSYTTLLLVLQYFGPLCFIFICYFKIYI-RL
HGRP   (160) KAA-FIWIISML-LAIPEAVFSDL   (194) ISC-AP-YPH   (208) KIHSMASFLVFYVIPLSIISVYYYFIAK-NL
HNMB   (163) KAM-GIWVVSVL-LAVPEAVFSEV   (195) TAC-IP-YPQ   (209) KIHSVLIFLVYFLIPLAIISIYYYHIAK-TL
HET1   (205) EIV-SIWILSFI-LAIPEAIFVMV   (236) KTC-ML-NAT   (252) DVKDWWLFGFYFCMPLVCTAIFYTLMTCEML
GPAF   (136) ALSLVIWVAIVA-AA-SYFLVMDS   (171) TRC-FEHYEK   (184) VLIIHICIVLGFFIVFLLILFCN-LVIIHTL
HPAF   (136) SLSLVIWVAIVG-AA-SYFLILDS   (171) TRC-FEHYEK   (184) VLIIHIFIVFSFFLVFLIILFCN-LVIIRTL
HC5A   (155) IACAVAWGLALL-LTIPSFLYRVV   (186) VLCGVD-YSH   (200) RAVAIVRLVLGFLWPLLTLTICYTFILLRTW
HIL8   (155) FVCLGCWGLSMN-LSLPFFLFRQA   (185) PVC-YEVLGN   (200) MVLRILPHTFGFIVPLFVMLFCYGFTLR-TL
HLPX   (145) VIV-GPWILALV-LTLPVFLFLTT   (174) TYCTFN-FAS   (199) TARGIIRFVIGFSLPMSIVAICYGLIAAKIH
HBRB2  (149) LYSLVIWGCTLL-LSSPMLVFRTM   (182) TACVIS-YPS   (195) VFTNMLLNVVGFLLPLSVITFC-TMQIMQVL
HAG2   (147) VTCIIIWLLAGL-ASLPAIIHRNV   (178) TVCAFH-YES   (193) IGLGLTKNILGFLFPFLIILTSYTLIW-KAL

MPGI   (268) LI-LL-ALMTVIMAVCSLPL-MIRGFTQAI   (304) LLAFRFNAFNPILDPW-VFILFRKAVFQR-L
HPGI   (238) LI-LL-ALMTVVMAVCSLPL-TIRCFTQAV   (275) LLAFRFYAFNPILDPW-VFILFRKAVFQR-L
MPGD   (263) FV-LL-ALMTVLFTMCSLPL-IYRAYYGAF   (302) LQALRFLSVISIVDPW-IFIIFRTSVFRM-L
HEP2   (263) LI-LL-AIMTITFAVCSLPF-TIFAYMNET   (298) LQALRFLSINSIIDPW-VFAILRPPVLRL-M
HEP4   (271) VI-LLIATS-LVVLICSIPL-VVRVFVNQL   (312) LQAIRIASVNPILDPW-IYILLRKTVLSKAI
MEP4   (299) VI-LLIATS-LVVLICSIPL-VVRVFINQL   (340) LQAIRIASVNPILDPW-IYILLRKTVLSKAI
REP4   (274) VI-LLIATS-LVVLICSIPL-VVRVFINQL   (315) LQAIRIASVNPILDPW-IYILLRKTVLSKAI
MTXA   (241) MVQLV-GIM-VVATVCWMPL-LVFIMQTLL   (288) LIYLRVATWNQILDPW-VYILFRRSVLRR-L
RTXA   (241) MVQLV-GIM-VVATVCWMPL-LVFILQTLL   (288) LIYLRVATWNQILDPW-VYILFRRSVLRR-L
HTXA   (244) MAQLL-GIM-VVASVCWLPL-LVFIAQTVL   (291) LIYLRVATWNQILDPW-VYILFRRAVLRR-L
BPGF   (248) VIQLL-GIM-CVSCICWSPF-LVTMASIGM   (287) LFTLRMATWNQILDPW-VYILLRKAVLRN-L
MPGF   (248) IIQLL-AIM-CVSCVCWSPF-LVTMANIAI   (287) LFALRMATWNQILDPW-VYILLRKAVLRN-L
RPGF   (248) VIQLL-AIM-CVSCVCWSPF-LVTMANIAI   (287) LFALRMATWNQILDPW-VYILLRKAVLRN-L
HEP1   (296) VGQLV-GIM-VVSCICWSPM-LVLVA-LAV   (333) FLAVRLASWNQILDPW-VYILLRQAVLRQ-L
MEP1   (299) VGQLV-GIM-VVSCICWSPL-LVLVV-LAI   (337) FLAVRLASWNQILDPW-VYILLRQAMLRQ-L
HEP3   (258) AIQLM-GIM-CVLSVCWSPL-LIMMLKMIF   (306) LIAVRLASLNQILDPW-VYLLLRKILLRK-F
RBEP3  (277) AIQLM-GIM-CVLSVCWSPL-LIMMLKMIF   (325) LIAVRLASLNQILDPW-VYLLLRKILLRK-F
MEP3   (257) AIQLM-GIM-CVLSVCWSPL-LIMMLKMIF   (305) LIAVRLASLNQILDPW-VYLLLRKILLRK-F
RTEP3  (257) AIQLM-GIM-CVLSVCWSPL-LIMMLKMIF   (305) LIAVRLASLNQILDPW-VYLLLRKILLRK-F
HSBP   (246) VVKMMIVVV-CTFAICWLPFHIFFLLP-YI   (290) IMWLAMSS-TM-YNPI-IYCCLNDR-FRLGF
HNMK   (296) VVKMMIIVV-MTFAICWLPYHIYFILT-AI   (340) SFWLAMSS-TM-YNPI-IYCCLNKR-FRAGF
HNPY   (261) INIMLLSIV-VAFAVCWLPLTIFNTVF-DW   (306) HL-TAMIS-TC-VNPI-FYGFLNKN-FQRDL
HGRP   (262) LAKTVLVFV-GLFAFCWLPNHVIYLYRSYH   (308) RL-LAFTN-SC-VNPFALY-LLSKS-FRKQF
HNMB   (263) LAKIVLVFV-GCFIFCWFPNHILYMYR-SF   (309) RV-LSFGN-SC-VNPFALY-LLSES-FRRHF
HET1   (303) VAKTVFCLV-VIFALCWFPLHLSRILKKTV   (353) GINLATMN-SC-INPIALY-FVSKK-FKNCF
GPAF   (230) ALWMVCTVL-AVFVICFVPHHMVQ-LPWTL   (278) TLCL-LST-NCVLDPV-IYCFLTKK-FRKHL
HPAF   (230) ALWMVCTVL-AVFIICFVPHHVVQ-LPWTL   (278) TLCL-LST-NCVLDPV-IYCFLTKK-FRKHL
HC5A   (240) TLKVVVAVV-ASFFIFWLPYQVTGIMM-S-   (285) CVSFAYIN-CC-INPI-IYVVAGQG-FQGRL
HIL8   (240) AMRVIFAVV-LIFLLCWLPYNLVLLAD-TL   (290) TEILGFLH-SC-LNPI-IYAFIGQN-FRHGF
HLPX   (239) PLRVLTAVV-ASFFICWFPFQLVALLG-TV   (287) TSSLAFFN-SC-LNPM-LYVFVGQD-FRERL
HBRB2  (241) ATVLVLVVL-LLFIICWLPFQISTFLD-TL   (290) ASFMAYSN-SC-LNPL-VYVIVGKR-FRKKS
HAG2   (238) IFKIIMAIV-LFFFFSWIPHQIFTFLD-VL   (287) TICIAYFN-NC-LNPL-FYGFLGKK-FKRYF
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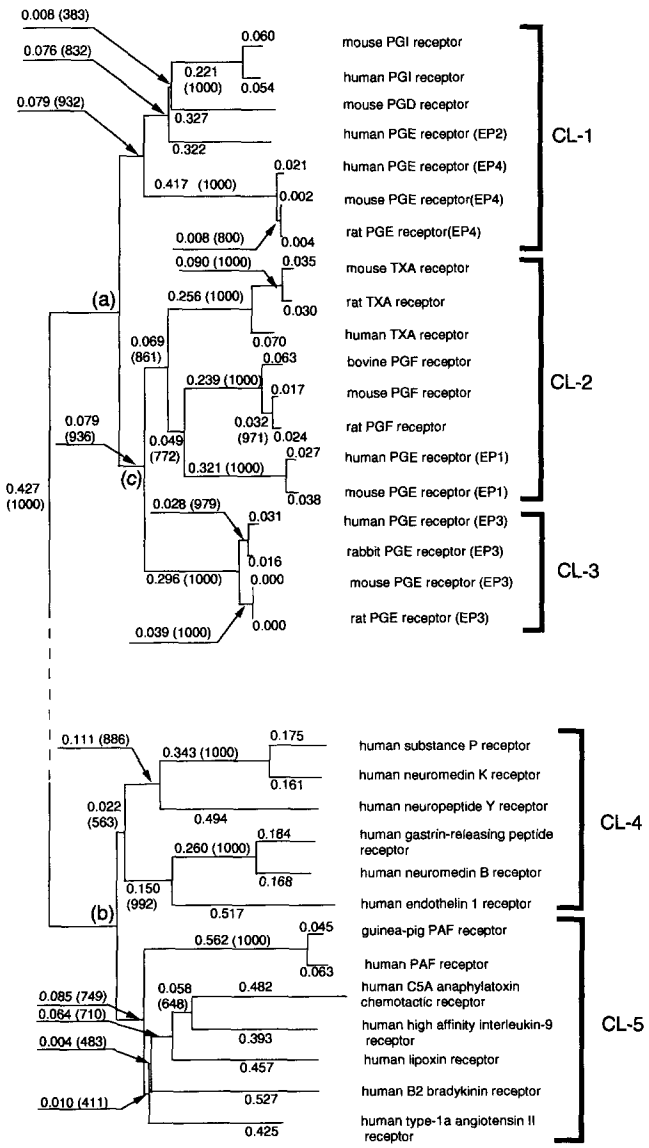

Fig. 2. A phylogenetic tree of the receptors for PGs, TXA, LXA, PAF and peptides. A number neighboring a branch or indicated by an arrow in the tree shows the reliability of the branch, that is, the count of success of the branch during one thousand iterations of bootstrap resampling and tree reconstruction. A branch with a number greater than or equal to nine-hundreds was regarded to be statistically significant.

The cluster consisting of peptide receptors included PAF and LXA receptors. The cluster was further divided into two subclusters, CL-4 and CL-5, at the node (b). CL-4 consisted of the receptors of neuropeptides and endothelin. The CL-5 included PAF receptors and a LXA receptor besides other peptide receptors. However, the origins of the two receptors for the lipid-derived ligands were different from each other. As shown in the tree, PAF receptors diverged at the early stage. Contrary to that, LXA receptor diverged more recently. Thus, it is suggested that the receptors for lipid-derived ligands have diverged from peptide receptors at least twice. In addition, the tree suggested that the origin of lipoxygenase pathway is different from that of cyclooxygenase pathway, that is, the two pathways have independently appeared and been integrated into the arachidonic acid cascade.

The outline on the evolutionary history of the arachidonic acid cascade was discussed with limited number of available sequences. Several problems has remained unknown. For example, the receptors involved in the lipoxygenase pathway may have diverged from an ancestral peptide receptor, although only LXA receptor has been sequenced in the receptors involved in the pathway. Further accumulation of the sequences for the receptors would ascertain the evolutionary history infered here and reveal more details for the evolution of the arachidonic acid cascade.

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