Biochemical Society Transactions www.biochemsoctrans.org

Non-genomic progesterone signalling and its non-canonical receptor

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Abstract

The steroid hormone progesterone regulates many critical aspects of vertebrate physiology. The nuclear receptor for progesterone functions as a ligand-activated transcription factor, directly regulating gene expression. This type of signalling is referred to as the 'genomic' pathway. Nevertheless, progesterone also stimulates rapid physiological effects that are independent of transcription. This pathway, termed 'non-genomic', is mediated by the mPRs (membrane progesterone receptors). These mPRs belong to a larger class of membrane receptors called PAQRs (progestin and adipoQ receptors), which include receptors for adiponectin in vertebrates and osmotin in fungi. mPRs have been shown to activate inhibitory G-proteins, suggesting that they act as GPCRs (G-protein-coupled receptors). However, PAQRs do not resemble GPCRs with respect to topology or conserved sequence motifs. Instead, they more closely resemble proteins in the alkaline ceramidase family and they may possess enzymatic activity. In the present paper, we highlight the evidence in support of each model and what is currently known for PAQR signal transduction of this non-canonical receptor.

Introduction

Progesterone is a steroid hormone that regulates female reproduction and other critical aspects of vertebrate physiology. Studies of progesterone signalling have mostly focused on the nuclear receptor, which functions as a progesterone-activated transcription factor and directly regulates gene expression [1]. This type of signalling is referred to as the 'genomic' pathway. However, progesterone also stimulates many rapid responses that are independent of transcription, such as changes in ion flux and intracellular Ca^{2+}, along with other second messengers [2]. This 'non-genomic' pathway is mediated by the mPRs (membrane progesterone receptors). mPRs have been identified not only in male and female reproductive tissues, but also in immune cells and tissues, the nervous system and cancer cells [2–6].

mPRs were first identified in fish ovaries [7,8], and a thorough review of the cloning and characterization of these receptors can be found in [2]. These mPRs belong to a larger class of membrane receptors called PAQRs (progestin and adipoQ receptors), which include receptors for adiponectin in vertebrates and osmotin in fungi [9]. The phylogenetic tree shown in Figure 1 demonstrates the relationship between the mPRs, PAQRs and other membrane proteins and shows that the mPR clade diverged from the rest of the PAQR family quite early in the evolution of metazoans, with representatives being found in simple placozoans. Thus progesterone signalling via the mPRs may precede signalling via the nuclear receptor [10] and may be the oldest form of steroid signalling in animals.

The predicted PAQR hydropathy shows seven TMs (transmembrane domains), comparable with GPCRs (G-protein-coupled receptors), and the signalling pathway they control seems to activate inhibitory G-protein cascades in female reproductive tissues [7,8,11–13]. However, this novel family of receptors lacks significant sequence similarity to any known GPCRs and may have opposite transmembrane topology. Moreover, a detailed sequence analysis of the PAQR family revealed that these receptors show sequence motifs characteristic of AlkCers (alkaline ceramidases) and present enzymatic activity as such [14,15]. A dispute for the true second messenger has occupied the literature in recent years and we highlight the evidence in support of each pathway.

Evidence for G-protein signalling

Even before the first mPR was cloned [7,8], it was suggested that progestin-dependent maturation of rainbow trout (*Oncorhynchus mykiss*) oocytes occurred via inhibitory G-proteins (G_i) [16]. It was observed that progestin treatment inhibited adenylate cyclase and lowered cAMP levels. Moreover, progestin increases non-hydrolysable GTP binding to membranes, whereas progestin binding decreases in the presence of non-hydrolysable GTP. PTX (*Pertussis* toxin), which prevents G_i from interacting with its receptor by preventing the exchange of bound GDP, was used to determine the identity of the G-protein involved. The same

Key words: ceramidase, G-protein, non-canonical receptor, progesterone, progestin and adipoQ receptor (PAQR), signalling.

Abbreviations used: AdipoR, adiponectin receptor; AlkCer, alkaline ceramidase; AMPK, AMP-activated protein kinase; APPL1, adaptor protein, phosphotyrosine interaction, pleckstrin homology domain and leucine zipper-containing 1; GPCR, G-protein-coupled receptor; mPR, membrane progesterone receptor; PAQR, progestin and adipoQ receptor; PTX, *Pertussis* toxin; S1P, sphingosine 1-phosphate; TM, transmembrane domain; TNFα, tumour necrosis factor α.

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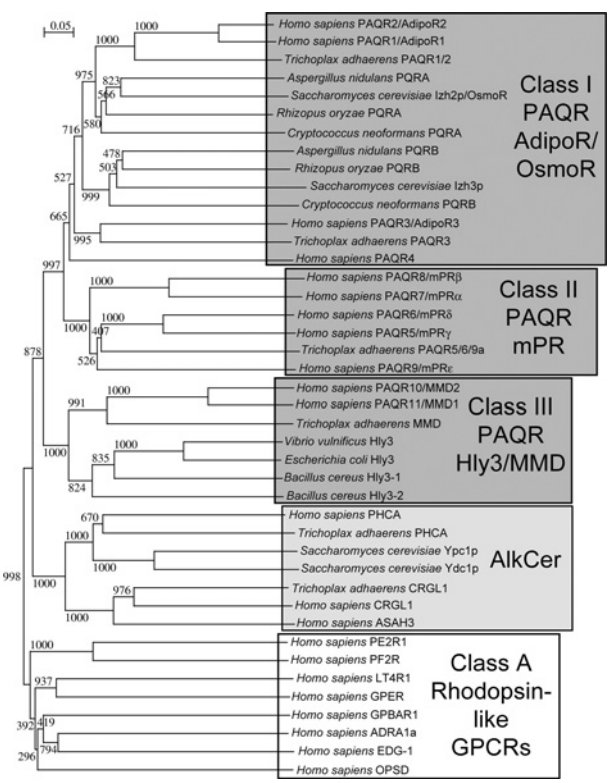
Figure 1 | Bootstrapped phylogenetic tree of PAQRs, AlkCers and GPCRs

The tree was generated by ClustalX using the neighbour-joining method with protein datasets truncated to include only the seven-TM core regions. Bootstrap values at the nodes are confidence values indicating the number of times per 1000 trees a particular grouping is made. Branch lengths are proportional evolutionary distance and the scale bar indicates the number of substitutions per site. Note: The AlkCers form a sister group to the PAQRs, whereas the human Class A GPCRs are highly divergent. The high bootstrap value at the node separating the human Class A GPCRs from PAQRs and AlkCers indicates a high degree of confidence in the inclusion of PAQRs and AlkCers into the same clade. However, the long branch lengths in the GPCR clade indicate that little unifies this family in terms of conserved sequence motifs. mPRs clearly form a group within the PAQR clade that we have termed Class II. The sister group to the mPRs is the clade containing the AdipoRs (Class I). Also, it is clear from this analysis that the simple placozoan *Trichoplax adhaerens* contains Class I, Class II and Class III PAQRs, suggesting the mPRs arose early in metazoan evolution. OsmoR, osmotin receptor.



was shown for Atlantic croaker (*Micropogonias undulatus*) oocytes [5], human myometrium [4], immortalized T-cells and leucocytes [3], and mouse hypothalamus and neuroblastoma cell lines [6].

Cloning of mPRs allowed expression in breast cancer cells lacking nuclear receptors, which facilitated non-genomic progestin signalling studies [7,8]. Stable transfections of fish and human mPRs in breast cancer cell lines displayed the same responses to non-hydrolysable GTP and PTX [13]. Moreover, the mPRα subtype could be detected in immunoprecipitated G_i pools, and this coupling was lost after progestin treatment. Co-immunoprecipitation of G_i and mPRs also has been shown in human myometrium [4]. However, transfections with two different mPR isoforms from zebrafish (*Danio rerio*) showed that both mPRα and mPRβ could decrease cAMP levels after progestin stimulation in a dose- and time-dependent manner, whereas only mPRα was affected by PTX [17].

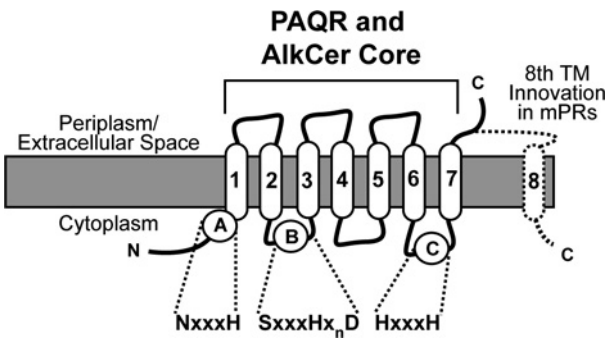
Most of the G-proteins associated with progestin non-genomic signalling to date have been of the inhibitory type. An exception worth noting is the activation of olfactory G-protein in Atlantic croaker sperm [18]. Progestin treatment increases cAMP concentrations in this system, and stimulation is prevented by *Cholera* toxin, which is an activator of stimulatory G-proteins as it prevents bound GTP from hydrolysing. Progestin treatment increases non-hydrolysable GTP binding to croaker membranes, and only olfactory G-proteins were detected, which co-immunoprecipitated with mPRα.

Despite the results described above, several findings have led to widespread unease with the categorization of mPRs as GPCRs. First, some groups have had difficulty confirming the activation of G_α proteins by mPRs [19]. Secondly, interactions have been inferred from co-immunoprecipitation assays of intact cellular membranes, which are likely to contain a heterogeneous mixture of membrane proteins. Thirdly, human mPRs can be functionally expressed in *Saccharomyces cerevisiae*, which does not express human G_α, and even functionally respond to progesterone in yeast cells lacking any endogenous G_α [10]. This suggests that G-proteins are not an essential component of PAQR-mediated signalling. That said, some studies suggest that G-proteins are important components of endogenous PAQR signalling in fungi [20,21], so the issue is not entirely resolved.

The only physical resemblance between GPCRs and PAQRs is the presence of a seven-TM core. In fact, phylogenetic analysis by Smith et al. [10] showed no apparent relationship between GPCRs and PAQRs. Rather, the phylogenetic tree in Figure 1 suggests that PAQRs are more similar to other seven-TM-containing protein families than they are to GPCRs. To complicate matters, the topology of this core in PAQRs is N_{in},C_{out} [22–24], rather than the characteristic N_{out},C_{in} topology of GPCRs. A model for the topology of PAQRs is shown in Figure 2. To be fair, not all GPCRs seem to conform to this characteristic topology [25], and it is even possible for some proteins to adopt multiple topologies [26]. Moreover, conflicting results have been published for the topology of proteins in the mPR subgroup, which possess an eighth TM C-terminal to the seven-TM PAQR core [10,13]. PAQRs and GPCRs do not share any conserved sequence motifs, but there are no conserved motifs that unify the entire GPCR family, a fact that has led some to speculate that the GPCR 'superfamily' is actually a polyphyletic group whose divergent members arrived at similar functions via homoplasy [27]. Ultimately, a canonical GPCR is defined as a receptor that sequesters transducer proteins (e.g. heterotrimeric G-proteins) at the membrane and releases them as second messengers upon binding of

Figure 2 | Topological model for PAQRs and AlkCers

A topology model for the PAQR family showing the seven-TM core, the N_{in},C_{out} topology and the three conserved motifs described in the text. The AlkCer family is characterized the by a seven-TM core with the same topology and the same three conserved motifs. The mPR subgroup of the PAQRs (also referred to as Class II PAQRs) are characterized by an additional TM that is C-terminal to the seven-TM core.



agonist. However, this definition is also troublesome, since there is evidence that some GPCRs do not absolutely require such transducers to initiate signalling [27]. In the end, there is little consensus as to what defines a GPCR other than the presence of the seven-TM core. Yet, enigmatically, not all proteins with a conserved seven-TM core are considered to be GPCRs (see the next section), complicating the unambiguous characterization of mPRs as GPCRs.

It is also important to note that G-proteins are not implicated in the signalling mechanism of AdipoRs (adiponectin receptors) [28]. The close relationship between AdipoRs and mPRs suggests that the two PAQR subfamilies may be functionally related, or at least work via similar signalling mechanisms. This is supported by the fact that both AdipoRs and mPRs can functionally couple to the same signal transduction pathway in yeast, suggesting a common mechanism of action [29]. Recently, Heiker et al. [28] succinctly reviewed what is known about the signalling pathway downstream of the AdipoRs. This involves dimerization of receptors, AMPK (AMP-activated protein kinase), APPL1 (adaptor protein, phosphotyrosine interaction, pleckstrin homology domain and leucine zipper-containing 1) and RACK1 (receptor for activated C-kinase 1). Some of these components are conserved in the signalling pathway in yeast (i.e. AMPK and APPL1) [29]. Unfortunately, the adiponectin and progesterone signalling research fields have largely proceeded in parallel without cross-talk. As the similarities between the AdipoRs and mPRs became more apparent, there is now a growing discussion of how the two fields may be interrelated [14,30].

Evidence for sphingolipid signalling

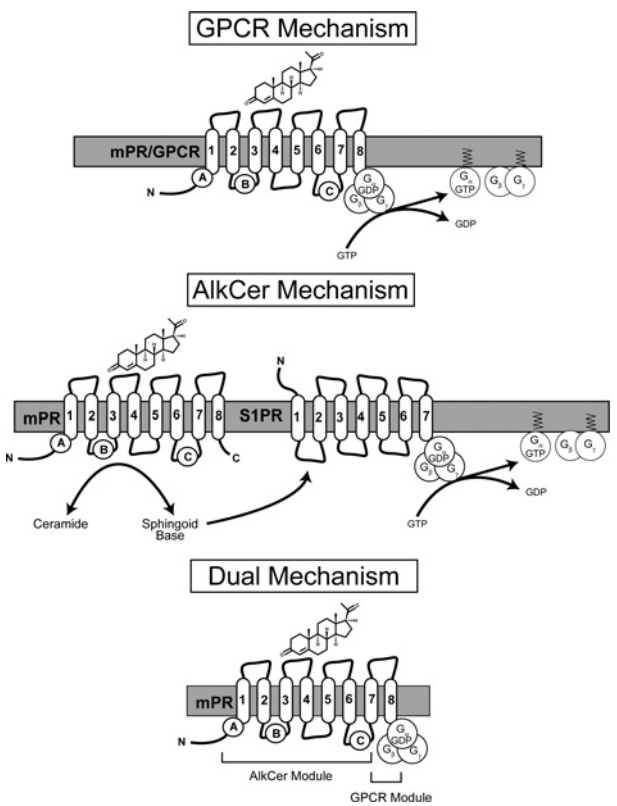
The unifying feature of the entire PAQR family is the presence of three highly conserved motifs at precise locations in the seven-TM core (Figure 2). Motif A (TM1) has the consensus NXXXH, Motif B (TM2–TM3) has the consensus SXXXHX$_n$D and Motif C (TM6–TM7) has the consensus HXXXH [10,15,31]. Intriguingly, these same three motifs can be found in exactly the same positions in another protein family that is characterized by a seven-TM core: the AlkCer [15] (also in Figure 2). In addition, the AlkCer family appears to have the same topology as the PAQR family [24]. AlkCers catalyse the reversible deacylation of ceramides to produce sphingoid bases. Both ceramides and sphingoid bases function as potent lipid second messengers in eukaryotic cells. The ratio of ceramides to sphingoid bases is often referred to as the ceramide rheostat, which globally regulates cellular metabolism [32]. The sequence similarity between the PAQRs and AlkCers suggests that the PAQRs might possess ceramidase activity. Indeed, a role for ceramides and sphingoid bases in PAQR signalling was first shown in yeast [15] and, subsequently, in mammalian cells [33]. Furthermore, whereas the addition of sphingoid base or overexpression of a known AlkCer recapitulates the effect of mPRs in the heterologous yeast expression system, the AlkCer inhibitor D-*erythro*-MAPP [(1*S*,2*R*)-D-*erythro*-2-(*N*-myristoylamino)-1-phenylpropan-1-ol] inhibits mPR signalling [14]. On the basis of these results and the similarities between the two families, it has been proposed that PAQRs might function as ligand-regulated AlkCer enzymes [15].

Although the ceramidase model for signal transduction via PAQRs was only recently proposed by Villa et al. [15], a role for sphingolipids in non-genomic progesterone signalling had already been established [34–37]. Indeed, in the oocyte maturation model of non-genomic progesterone signalling, the addition of sphingoid base can replace progesterone as the inducer of germinal vesicle breakdown [37]. Intriguingly, the AlkCer family of enzymes seems to catalyse both ceramidase and ceramide synthase reactions [38], and it is possible that the PAQRs might possess both activities as well. It has been proposed that PAQRs might be in the unique position to control many aspects of cellular metabolism as a fulcrum for the ceramide rheostat, depending upon whether or not ceramidase or ceramide synthase activity is favoured [14]. In support of this model, both agonists (progestins) and inverse agonists [TNFα (tumour necrosis factor α)] have been identified for the mPRs using the yeast system as a functional assay. TNFα is a homologue of adiponectin that is known to generate ceramide second messengers. It is known that TNFα counteracts the effects of both progesterone and adiponectin, therefore the mPR and AdipoR families may be unified by their antagonism to TNFα [14].

The established role of G_i in progesterone signalling described above, as well as some other non-genomic effects of progesterone, could alternatively be explained as a downstream effect of sphingolipid signalling. Phosphorylated sphingoid bases [S1P (sphingosine 1-phosphate)] are known activators of receptors in the EDG subfamily of GPCRs and the S1P receptors are known to couple to G_i [39]. Since both PAQRs and S1P receptors are involved in sphingolipid signalling, it would not be surprising if they co-localized to membrane microdomains or 'lipid rafts' that are enriched

Figure 3 | Signal transduction models

Two distinct models for the mechanism of signalling via mPRs have been proposed. In the GPCR model, the mPRs function as GPCRs that sequester inactive G-proteins and release them upon binding of progesterone. In the AlkCer model, the mPRs function as AlkCer enzymes that interconvert ceramides and sphingoid bases, which function as potent second messengers, including in the activation of the EDG-1 S1P receptor, a known GPCR. Since mPRs are distinguished from other PAQRs by the presence of an eighth TM at the C-terminus, we have proposed a third 'dual' model where the seven-TM PAQR core functions as an AlkCer, whereas the C-terminus unique to mPRs adds GPCR functionality.



in sphingolipids. Indeed, this seems to be true for the EDG-1 S1P receptor [40] as well as for the yeast PAQR Izh2p (N. Villa and T. Lyons, unpublished work). Co-localization of mPRs and a S1P receptor to detergent-resistant microdomains might also explain co-immunoprecipitation results, suggesting an association between G_i and the mPRs.

The best of both worlds

We have suggested that G_i binding to the mPRs, and therefore the GPCR model for signalling, may be an artefact of the co-immunoprecipitation assay used. However, this need not be the case. Indeed, the GPCR and AlkCer models for PAQR signalling are not necessarily mutually exclusive (Figure 3). As mentioned above, the mPRs differ from all other PAQRs by the presence of an eighth TM C-terminal to the seven-TM PAQR core. The function of this additional module is unknown, and the fact that mPRs respond normally to progesterone in the functional yeast assay system even without this domain suggests that it is not involved in progesterone binding [10]. However, Thomas et al. [13] did find that this additional module is important for G-protein binding to membranes. Therefore this module, which is unique to the mPRs, may add GPCR functionality on top of AlkCer activity by providing a docking site for heterotrimeric G-proteins, allowing dual sphingolipid/G-protein signal transduction pathways in response to progesterone (Figure 3). That the module is not required for progesterone-dependent signalling in the functional yeast assay system may be explained if the yeast assay only measures output from the PAQRs to the sphingolipid branch of the downstream pathway, leaving the G-protein branch unmonitored. It is possible that the published work in yeast tracks only the sphingolipid aspect of the signal transduction pathway, which is common to all PAQRs, whereas the published work in vertebrate cells has been tracking the G-protein component that is unique to mPRs. It is also possible that, like some phospholipases [41], the PAQRs function as downstream effectors of G-protein signalling whose activity is regulated by G-protein binding.

Funding

Our work is funded by the National Institutes of Health [grant numbers R01 GM086617-01 and R21 DK074812 (to T.J.L.)].

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Received 14 July 2011
doi:10.1042/BST20110638