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A computational analysis of non-genomic plasma membrane progestin binding proteins: Signaling through ion channel-linked cell surface receptors ☆

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ABSTRACT

A number of plasma membrane progestin receptors linked to non-genomic events have been identified. These include: (1) α1-subunit of the Na^+/K^+-ATPase (ATP1A1), (2) progestin binding PAQR proteins, (3) membrane progestin receptor alpha (mPRα), (4) progesterone receptor MAPR proteins and (5) the association of nuclear receptor (PRB) with the plasma membrane. This study compares: the pore-lining regions (ion channels), transmembrane (TM) helices, caveolin binding (CB) motifs and leucine-rich repeats (LRRs) of putative progesterone receptors. ATP1A1 contains 10 TM helices (TM-2, 4, 5, 6 and 8 are pores) and 4 CB motifs; whereas PAQR5, PAQR6, PAQR7, PAQRB8 and fish mPRα each contain 8 TM helices (TM-3 is a pore) and 2–4 CB motifs. MAPR proteins contain a single TM helix but lack pore-lining regions and CB motifs. PRB contains one or more TM helices in the steroid binding region, one of which is a pore. ATP1A1, PAQR5/7/8, mPRα, and MAPR-1 contain highly conserved leucine-rich repeats (LRR, common to plant membrane proteins) that are ligand binding sites for ouabain-like steroids associated with LRR kinases. LRR domains are within or overlap TM helices predicted to be ion channels (pore-lining regions), with the variable LRR sequence either at the C-terminus (PAQR and MAPR-1) or within an external loop (ATP1A1). Since ouabain-like steroids are produced by animal cells, our findings suggest that ATP1A1, PAQR5/7/8 and mPRα represent ion channel-linked receptors that respond physiologically to ouabain-like steroids (not progestin) similar to those known to regulate developmental and defense-related processes in plants.

1. Introduction

Increasing evidence suggests that a number of steroid-induced events are initiated in animal cells independent of transcription (reviewed in [1–6]). As outlined by Watson and Gametchu [2], the presence of steroid receptors in the cell membrane contributes to the regulation of a wide range of complex functions, including cell proliferation, cell death and cell differentiation. The identification of plasma membrane receptors has often been based either on radiolabeled ligand binding to crude plasma membrane-enriched preparations or on a putative steroid binding protein obtained by over-expression in a bacterial system. Mitochondria also contain membrane-bound steroid receptors similar or identical to the corresponding nuclear receptor (reviewed in [7]).

This study analyzes several putative progestin binding membrane receptors with respect to: transmembrane helices and pore-lining membrane regions (ion channels) [8], caveolin binding motifs [9], and the highly conserved leucine-rich repeats associated with steroid binding in plants [10,11]. Computational analysis was used to compare putative steroid receptors in terms of their structural similarities and possible common ancestors in animal and plant cells. The putative animal cell progestin-binding proteins entered in the protein data base (UniprotKB) consist of proteins containing 195–1023 amino acid residues. These can be divided into 5 groups: (1) the catalytic (alpha) subunits of Na/K-ATPase [12], (2) at least three members of an 11 membrane receptor family termed "PAQR" proteins (reviewed in [13]), (3) several proteins termed "membrane progestin receptors alpha, beta and gamma" (reviewed in [14]), (4) the "membrane-associated progesterone receptor" components 1 and 2 belonging to the MAPR family (reviewed in [15]), and (5) the progesterone nuclear receptor (PRB) which may associate to the plasma membrane via its ligand binding domain [16].

Na^+/K^+-ATPase is a αβ-tetramer, with 2 binding sites for the plant steroid, ouabain, on each α-subunit (reviewed in [17]). It

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should be emphasized that plasma membrane Na^+/K^+-ATPase not only regulates intracellular Na^+ and K^+, levels, but also serves as a critical cell signaling component [18]. PAQR receptors play a role in signal transduction for a wide range of ligands, including steroids, lipids, nucleotides, peptides and photons (reviewed in [19]). PAQR5, PAQR7 and PAQR8 have been defined as membrane progestin receptor γ, α, and β, respectively [19], but PAQR7 is best characterized [20]. Several so-called MAPR proteins have been identified (reviewed in [21]). MAPR proteins contain cytochrome b5-like heme/steroid-binding domains and include progesterone component 1 (PGRMC1), progesterone component 2 (PGRMC2), neudesin and neuferricin.

Classical estrogen, progesterone and androgen receptors are thought to be tethered to the inside of the plasma membrane and thus localized outside the nucleus (reviewed in [22]). Many progesterone effects are mediated by a nuclear receptor that is expressed as two isoforms, PRA and PRB, which are virtually identical except that PRA lacks 164 amino acids (in humans) in the N-terminal region (reviewed in [23]). *In vitro* evidence suggests that the A and B forms are functionally distinct and distribution patterns may account for some of the diversity of progesterone effects. Photoaffinity labeling studies with *Rana pipiens* oocytes indicate PRB (but not PRA) is equally distributed between plasma membranes and cytosol, suggesting that PRB may function in both compartments during progesterone-induced oocyte meiosis [24]. Martinez et al. [16] found that overexpression or depletion of the nuclear receptor PRB in *Xenopus laevis* oocytes can accelerate or block progesterone-induced oocyte maturation. Additional evidence indicates that the nuclear receptor may associate with cytoplasmic factors (e.g. p42 MAPK) contributing to non-genomic signaling [25]. Using newer algorithms, we report here that both PRA and PRB contain at least 1 pore-lining TM helix localized to the steroid binding region, suggesting that membrane PRB may also be anchored by a single transmembrane helix and serves as a specialized channel.

Our analysis indicates that, except for PGRMC1/2, the progestin binding proteins contain pore-lining regions within specific transmembrane helices that may function as ion channels. All but PMRMC1/2 and the classical cytosol (nuclear) receptor (PR) contain both caveolin binding motifs and the highly conserved leucine-rich repeats (LRR) common to plant steroid receptors (reviewed in [11]). Plants produce numerous steroids and sterols (now found to act as hormones in animals [26]), but lack close homologues of animal nuclear steroid receptors (Arabidopsis Genome Initiative). In plant cells, polyhydroxylated steroids bind to leucine-rich sequences in membrane receptors and elicit responses even in the presence of inhibitors of transcription or protein synthesis (cit. [11]), similar to the progesterone/polar steroid induction of the meiotic division in vertebrate oocytes [27]. This suggests that plant and animal cells may have retained a common steroid hormone-membrane receptor response system during evolution. The protein structure–function studies outlined here indicate that many progestin binding proteins are concentrated in caveolin-rich plasma membrane lipid rafts and function both as ion channels and as receptors for polyhydroxylated (ouabain-like) steroids.

2. Experimental

2.1. Protein sequence sources

The amino acid sequences of the α1-subunit of Na/K-ATPase and putative progestin binding proteins were downloaded from the ExPASy Proteonomic Server of the Swiss Institute of Bioinfomatics (http://www.expasy.org; http://www.uniprot.org). About 98% of the protein sequences provided by UniProtKB are derived from the translation of coding sequences (CDS) which were submitted to the public nucleic acid databases, the EMBL-Bank/Genbank/DDBJ databases (INSDC). Data shown are for humans with the exception of membrane receptor alpha for the Atlantic croaker. Amino acid sequences were compared using the Pairwise Sequence Alignment software (LALIGN) at http://www.ebi.ac.uk/Tools/services/weblalign/ to find internal duplications by calculating non-intersecting local alignments [28]. The Emboss Water protocol (version 36.3.5e Nov, 2012; preload8) used here employs the Smith–Waterman algorithm (modified enhancements) to calculate the local alignment of two sequences.

2.2. Secondary structure predictions

Secondary structures were predicted by PSIPRED v.3.0; http://bioinf.cs.uci.ac.uk/psipred/ [29] and PredictProtein; http://predictprotein.org [30]. The PSIPRED secondary structure prediction method is based on position-specific scoring matrices. PredictProtein provides multiple sequence alignments and predictions of secondary structure, residue solvent accessibility and the location of TM helices (http://rostlab.org/owiki/index.php/PredictProtein).

2.3. Transmembrane (TM) helix and pore-lining region predictions

TM helices were predicted using: (1) the TOPCONS algorithm [31]; (http://topcons.cbr.su.se), (2) Phobius: (predicts TM topology and signal peptides), (http://phobius.cgb.ki.se and http://phobius.btnf.ku.dk/) European Biomathematics Institute [32], (3) PredictProtein [30]; (http://ebi.ac.uk/~rost/predictprotein), (4) the MEMSAT-SVM server [29], (5) SPOCTOPUS [33], a combined predictor of signal peptides and membrane topology (http://octopus.cbr.su.se/) and (6) TMHMM [34], based on a hidden Markov model available at: http://www.cbs.dtu.dk/services/TMHMM/. Pore-lining regions in transmembrane protein sequences were predicted using the method of Nugent and Jones [35]. The helical wheel applet on the University of Virginia web site was used: (http://cti.itc.virginia.edu/).

2.4. Caveolin-binding motifs

Using a GST-fusion protein containing the caveolin scaffolding domain as a receptor to select peptide ligands from a bacteriophage display library, Couet et al. [9] identified at least two related but distinct caveolin binding motifs, ΦxxxxΦxxΦ and ΦxΦxxxxΦ (where Φ represents an aromatic amino acid, W, Y, or F), in most proteins that had been shown to interact with caveolin.

3. Results and discussion

3.1. Comparison of putative progestin-binding plasma membrane receptor proteins

Table 1 compares structural properties of representatives of five groups of putative progestin-binding membrane proteins: (1) the α1-subunit of human Na^+/K^+-ATPase (P05023), (2) the human AdipoQ receptor family members PAQR5 (Q9NXK6), PAQR6 (Q6TCH4), PAQR7 (Q86WK9) and PAQR8 (Q8TEZ7), (3) membrane progestin receptor alpha (mPRα) from Atlantic croaker (A7XS19), (4) human membrane-associated progesterone receptor (MAPR) identified as Component-1 (O00264) and Component-2 (O15173), plus (5) the classic human nuclear progesterone receptor PR-B (P06401). The nomenclature of putative membrane-associated progestin-binding proteins is often confusing. For example, there are 11 members of the PAQR family and 3 PAQR proteins (5, 7, and 8) have been defined as progestin receptors γ, α and β, respectively. MAPR-1 is also