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Molecules in focus

Phosphorylation mediated structural and functional changes in pentameric ligand-gated ion channels: Implications for drug discovery

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ABSTRACT

Pentameric ligand-gated ion channels (pLGICs) mediate numerous physiological processes, including fast neurotransmission in the brain. They are targeted by a large number of clinically-important drugs and disruptions to their function are associated with many neurological disorders. The phosphorylation of pLGICs can result in a wide range of functional consequences. Indeed, many neurological disorders result from pLGIC phosphorylation. For example, chronic pain is caused by the protein kinase A-mediated phosphorylation of α3 glycine receptors and nicotine addiction is mediated by the phosphorylation of α4- or α7-containing nicotinic receptors. A recent study demonstrated that phosphorylation can induce a global conformational change in a pLGIC that propagates to the neurotransmitter-binding site. Here we present evidence that phosphorylation-induced global conformational changes may be a universal phenomenon in pLGICs. This raises the possibility of designing drugs to specifically treat disease-modified pLGICs. This review summarizes some of the opportunities available in this area.

1. Introduction

The pentameric ligand-gated ion channel (pLGIC) family includes nicotinic acetylcholine receptors (nAChRs), $GABA_A$ receptors ($GABA_A$Rs), glycine receptors (GlyRs) and 5-HT_3 receptors (5-HT_3Rs). Although these receptors are involved in many physiological processes, they are probably best known for mediating fast neurotransmission in the nervous system. They have been implicated in numerous channelopathies with neurological manifestations and are pivotal pharmacological targets. For example, nAChRs play a clinical role in nicotine addiction and are therapeutic targets for Alzheimer's disease and schizophrenia (Pollock et al., 2009); $GABA_A$Rs are important clinical targets for epilepsy (Abramian et al., 2010), anxiety, addiction and anaesthesia (Song and Messing, 2005); GlyRs are emerging targets for chronic inflammatory pain (Harvey et al., 2004).

Phosphorylation is well known to influence synaptic function by directly modulating pLGICs (Swope et al., 1999). Indeed, pLGIC phosphorylation has been implicated in various disorders such as nicotine addiction (Wecker et al., 2001), status epilepticus (Terunuma et al., 2008) and chronic pain (Harvey et al., 2004). Phosphorylation of pLGICs can elicit a wide variety of effects, ranging from alterations in the level of surface expression, synaptic targeting and receptor desensitization. Many of these effects are summarized in Table 1. Until recently, there have been few attempts to resolve the structural basis of these effects. By doing so, it may be possible to design drugs to specifically treat the consequences of pathological receptor modifications that lead to disease. This review summarizes some of the opportunities available in this area.

2. Structure

2.1. pLGIC architecture

Functional pLGICs comprise pentameric assemblies of identical or different subunits (Fig. 1A). The five subunits together form a central water-filled pore that facilitates transmembrane ion flux (Fig. 1B). Each subunit can be divided into three domains, an extracellular ligand-binding domain, four transmembrane helices (termed M1–M4) and a large intracellular M3–M4 domain which

Abbreviations: 5-HT_3R, 5-hydroxytryptamine type-3 receptor; CamKII, Ca^{2+}/calmodulin-dependent protein kinase; $GABA_A$R, GABA type-A receptor; GlyR, glycine receptor; IPSC, inhibitory postsynaptic current; nAChR, nicotinic acetylcholine receptor; PKA, protein kinase A; PKC, protein kinase C; pLGIC, pentameric ligand-gated ion channel; PTK, protein tyrosine kinase; PGE_2, prostaglandin type-E_2; VCF, voltage clamp fluorometry.

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Table 1
Effect of phosphorylation on pLGICs and their relevance to diseases.

Receptor	Subtype	Phosphorylation site	Protein kinase	Effect on receptor function	Disease relevance	References
GlyR	α3	S346	PKA	Inhibition of glycinergic currents	Chronic inflammatory pain	Harvey et al. (2004)
	β1	S409	PKA, PKC	Enhanced desensitization and prolonged deactivation	Epilepsy	Hinkle and Macdonald (2003)
	β3	S408, S409	PKC	Alterations in cell surface expression.	Status epilepticus	Terunuma et al. (2008)
			PKC	BDNF-mediated transient increase in receptor function followed by down regulation	Neuronal Excitotoxicity	Jovanovic et al. (2004)
		S383	CamKII	Rapid insertion of extrasynaptic receptors at the cell surface and enhanced tonic currents in hippocampus	Neuropsychiatric disorders	Saliba et al. (2012)
$GABA_AR$	γ2	S327	PKC	Alterations in ethanol and benzodiazepine sensitivity	Alcoholism	Qi et al. (2007)
		S343	PKC	Increased amplitude of mIPSCs	Alcoholism	Song and Messing (2005)
		Y365, Y367	Src	Increased postsynaptic receptor expression and enhanced frequency of hippocampal mIPSCs	Spatial memory deficits	Tretter et al. (2009), Luscher et al. (2011)
	α4	S443	PKC	Increased cell surface stability and activity	Temporal lobe epilepsy	Abramian et al. (2010)
	α7	Y386, Y442	Src	Increased current amplitudes with no change in surface receptor number	Cognition and addiction	Charpantier et al. (2005)
		Y386, Y442, Y317	Src	Increased surface receptor numbers with no change in open probability		Cho et al. (2005)
nAChR (neuronal)	α4	S368	PKC	Prolonged nicotine-induced desensitization	Nicotine addiction	Wecker et al. (2001)
		S365, S472, S491	PKA	Increased surface expression		Guo and Wecker (2002)
		S467	PKA	Favoured expression of low-affinity receptors		Bermudez and Moroni (2006), Pollock et al. (2009)
		S550	PKC	Dual action affecting mature receptors to stabilize the receptors at cell surface and increase transport of immature receptors from endoplasmic reticulum to membrane		Pollock et al. (2009)
	β	Y390	Src	Decreased receptor turnover and metabolic stabilisation	Myasthenia gravis	Rudell and Ferns (2013)
		Y355	Src	Enhanced desensitization and immobilization of the receptor		Tzartos et al. (1993)
nAChR (muscle)	δ	S361, S362	PKA	Enhanced desensitization	Myasthenia gravis	Hoffman et al. (1994)
		Y372	Src	Stable localisation of the receptor during synaptogenesis		Wagner et al. (1991)
	γ	S353, S354	PKA	Enhanced desensitization		Hoffman et al. (1994)

has a length and primary structure that varies enormously among pLGIC members (Fig. 1A). The extracellular domain predominantly comprises a twisted β-sheet sandwich with ligand-binding pockets located at the subunit interfaces. The principal (+) side of the pocket is lined by binding domain loops A, B and C and the complementary (−) side is lined by binding loops D, E and F (Fig. 1A). The transmembrane α-helices form concentric rings around a central ion pore, which is directly lined by five M2 helices (Fig. 1A).

2.2. Structure and importance of the M3–M4 cytoplasmic domain

Unlike the extracellular and transmembrane domains, the M3–M4 domain is poorly conserved both in terms of length and amino acid sequence. Therefore, it is likely to exhibit structural variation. Low-resolution structural data suggest that the *Torpedo* nAChR M3–M4 domain forms a 'hanging basket' type structure connecting the pore with the cytoplasm (Miyazawa et al., 1999). This structure incorporates a lateral ion permeation pathway (or portal) linking the cytoplasm with the inner vestibule at the base of the pore. Charged residues lining these portals influence the single channel conductance of nAChRs, GlyRs and $5HT_3Rs$ (Peters et al., 2010). Interactions between the M3–M4 loop and other proteins or ions are well known to modulate pLGIC activity, assembly and trafficking (e.g., Luscher et al., 2011). These interactions are highly specific to different pLGIC subunits. The M3–M4 domain is also the only pLGIC region known to house phosphorylation sites.

2.3. Receptor phosphorylation: structural changes induced by kinases?

Phosphorylation results from the kinase-mediated covalent attachment of the γ-phosphate group of ATP to the hydroxyl group of serine, threonine or tyrosine (Fig. 1D). The best-characterized protein kinases include cAMP dependent protein kinase A (PKA), protein kinase C (PKC) and protein tyrosine kinase (PTK). Of these, PKA and PKC phosphorylate both Ser and Thr residues and PTK (including Src family kinase) phosphorylates Tyr residues. Many other kinases also exist such as Ca^{2+}/calmodulin-dependent