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Potentiation and allosteric agonist activation of α7 nicotinic acetylcholine receptors: binding sites and hypotheses☆

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ABSTRACT

Considerable progress has been made in recent years towards the identification and characterisation of novel subtype-selective modulators of nicotinic acetylcholine receptors (nAChRs). In particular, this has focussed on modulators of α7 nAChRs, a nAChR subtype that has been identified as a target for drug discovery in connection with a range of potential therapeutic applications. This review focusses upon α7-selective modulators that bind to receptor sites other than the extracellular 'orthosteric' agonist binding site for the endogenous agonist acetylcholine (ACh). Such compounds include those that are able to potentiate responses evoked by orthosteric agonists such as ACh (positive allosteric modulators; PAMs) and those that are able to activate α7 nAChRs by direct allosteric activation in the absence of an orthosteric agonist (allosteric agonists or 'ago-PAMs'). There has been considerable debate about the mechanism of action of α7-selective PAMs and allosteric agonists, much of which has centred around identifying the location of their binding sites on α7 nAChRs. Based on a variety of experimental evidence, including recent structural data, there is now clear evidence indicating that at least some α7-selective PAMs bind to an inter-subunit site located in the transmembrane domain. In contrast, there are differing hypotheses about the site or sites at which allosteric agonists bind to α7 nAChRs. It will be argued that the available evidence supports the conclusion that direct allosteric activation by allosteric agonists/ago-PAMs occurs via the same inter-subunit transmembrane site that has been identified for several α7-selective PAMs.

1. Introduction

Nicotinic acetylcholine receptors (nAChRs) are oligomeric transmembrane proteins that are activated by the binding of the neurotransmitter acetylcholine (ACh). They are also members of a larger family of pentameric ligand-gated ion channels (pLGICs) that includes receptors for several other neurotransmitters, including 5-hydroxytryptamine, γ-aminobutyric acid and glycine [1,2]. A variety of experimental approaches have provided evidence that, in common with other pLGICs, nAChRs are assembled from five transmembrane subunits; each of which is a single polypeptide containing an N-terminal extracellular domain, four transmembrane α-helices (M1-M4) and an intracellular domain formed largely by the region between the M3 and M4 helices (Fig. 1) [3]. Five nAChR subunits assemble in a ring-like structure to create a central cation-selective ion channel pore and agonist activation of cell-surface nAChRs is associated with a conformational change resulting in the opening of the ion-channel pore and an influx of cations into the cell.

There are sixteen different nAChR subunits in humans (α1-α7, α9, α10, β1-β4, γ, δ and ε) and these can assemble into a wide variety of nAChR subtypes with differing subunit composition [4]. Most nAChRs are 'heteromeric' (containing more than one type of subunit), however there are restrictions on which nAChR subunits can productively co-assemble with which others to produce functional pentameric receptors. Nevertheless, there is a considerable degree of heterogeneity in terms of subunit composition, particularly amongst nAChR subtypes that are located within the central nervous system [4]. Amongst the sixteen human nAChR subunits, the α7 subunit is unusual in that it is capable of assembling into a functional nAChR containing five copies of the same subunit (a 'homomeric' nAChR) [5]. Homomeric α7 nAChRs are also unusual in that they display very rapid desensitisation following

Abbreviations: ACh, acetylcholine; cryo-EM, cryogenic electron microscopy; MLA, methyllycaconitine, PAM, positive allosteric modulator; PDB, protein databank; pLGIC, pentameric ligand-gated ion channel; nAChR, nicotinic acetylcholine receptor, NAM, negative allosteric modulator; SAM, silent allosteric modulator.

☆ The multifaceted activities of nervous and non-nervous neuronal nicotinic acetylcholine receptors in physiology and pathology. Eds: Dr Cecilia Gotti, Prof Francesco Clementi, Prof Michele Zoli

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activation by the endogenous agonist, ACh [5]. Indeed, desensitisation of α7 nAChRs in response to activation by ACh is so rapid that when a population of receptors is examined by standard electrophysiological techniques, desensitisation begins long before all of the receptors in the population have been activated. Homomeric α7 nAChRs have also been implicated in a range of neurological disorders and this has led to a considerable amount of interest in understanding the relationship between its structure and function, and in identifying novel α7-selective ligands [6]. In addition to its ability to form homomeric receptors, the nAChR α7 subunit is also able to co-assemble with β2 to form heteromeric α7β2 nAChRs, resulting in slower desensitisation kinetics and altered pharmacological properties [7]. In addition, in humans, the exons 5–10 of the gene encoding the α7 subunit (*CHRNA7*) is duplicated and fused to *FAM7A* to produce a hybrid gene (*CHRFAM7A*), producing a truncated subunit, lacking the extracellular N-terminal domain (dupα7) [8] and there is evidence that the α7 subunit can co-assemble with dupα7, resulting in altered functional properties [9].

1.1. Orthosteric and allosteric binding sites

A common feature of pLGICs is that they can be modulated by a variety of ligands that bind to multiple distinct binding sites. To reflect this, it has become common for the well-defined extracellular agonist binding to be described as the 'orthosteric site', thereby distinguishing it from other sites on pLGICs at which ligand binding can modulate receptor function ('allosteric sites'). The nAChR orthosteric binding site is located in the extracellular domain at the interface of adjoining subunits. Indeed, its precise location is very well defined, having been confirmed by the determination of several high-resolution structures of nAChRs containing bound orthosteric agonists [10–13]. Ligands that are capable of influencing receptor function by binding at a location other than the orthosteric site are classified as allosteric modulators and can mediate a range of pharmacological effects. There can, however, be some inconsistency about whether certain nAChR binding sites are considered to be 'orthosteric' or 'allosteric' sites, particularly in heteromeric receptors. In a homomeric nAChR, such as α7, there are five extracellular orthosteric binding sites located at the interfaces between adjacent subunits, all of which are capable of binding ACh [14]. However, as has been discussed in detail elsewhere [15], during the evolution of heteromeric nAChRs (which is presumed to have occurred from homomeric ancestors), the analogous binding sites at some subunit interfaces may have lost the ability to bind ACh. Binding of ligands other than ACh to such 'unorthodox orthosteric sites' or 'non-canonical sites' in heteromeric receptors can potentially influence responses to ACh mediated at conventional orthosteric site [15]. The effects mediated by such sites are sometimes described as allosteric modulation, however it is probably more common for allosteric modulators to be viewed as ligands that act at sites other than at these structurally- and evolutionarily-related orthosteric, unorthodox or non-canonical extracellular sites.

2. Allosteric modulators

Allosteric modulators of nAChRs that lack intrinsic agonist activity but which are able to potentiate responses evoked by orthosteric agonists are commonly referred to as positive allosteric modulators (PAMs) but allosteric modulators also include ligands that can inhibit agonist-evoked responses, such as non-competitive antagonists or negative allosteric modulators (NAMs) [16]. The term silent allosteric modulator (SAM) has also been used to describe compounds that have no obvious effect on responses evoked by orthosteric agonists but which can block the effects of allosteric modulators such as PAMs or NAMs [17]. Allosteric agonists comprise a further class of allosteric modulator and are compounds that are able to cause direct activation of the receptor but which do so by binding to a site that is distinct from the conventional orthosteric site. All such compounds that have been identified as allosteric agonists of α7 nAChRs also act as potentiators (PAMs) of responses to orthosteric agonists and, as a consequence, have also been referred to as 'ago-PAMs'.

A considerable amount of interest has focussed around the identification of novel α7-selective PAMs and these are frequently classified as either 'type-I' or 'type-II' PAMs. While both of these types of PAM typically increase agonist efficacy, as illustrated by increased peak agonist-evoked responses, type-II PAMs cause a marked reduction in the normally very rapid agonist-induced desensitisation of α7 nAChRs (Fig. 2) [16,18,19]. In addition, type-II PAMs (but not type-I) are able to facilitate reactivation of α7 nAChRs from a desensitised state [16,19,20]. However, this classification of 'type-I' and 'type-II' PAMs is an over-simplification, since compounds with intermediate effects have also been identified [16,21,22]. Nevertheless, this terminology has been retained in the present article, since it is in widespread use and there are situations where the distinction can be useful.

2.1. Positive allosteric modulators

Several compounds have been identified that potentiate α7 nAChRs but which are relatively non-selective (in the sense that they also modulate other nAChR subtypes or other pLGICs). Examples include small molecules such as 5-hyroxyindole [23] and large macrocyclic lactones such as ivermectin [24]. There is also evidence of potentiation of α7 nAChRs by proteins such as serum albumin [25]; the tyrosine kinase inhibitor, genistein [19] and the Ly-6/uPAR-related protein, SLURP-1 [26]. However, there has been considerable interest in the development of PAMs that display either higher potency or greater specificity for α7 nAChRs. It is a goal that has been motivated, at least in part, as a consequence of α7 nAChRs having been identified as potential

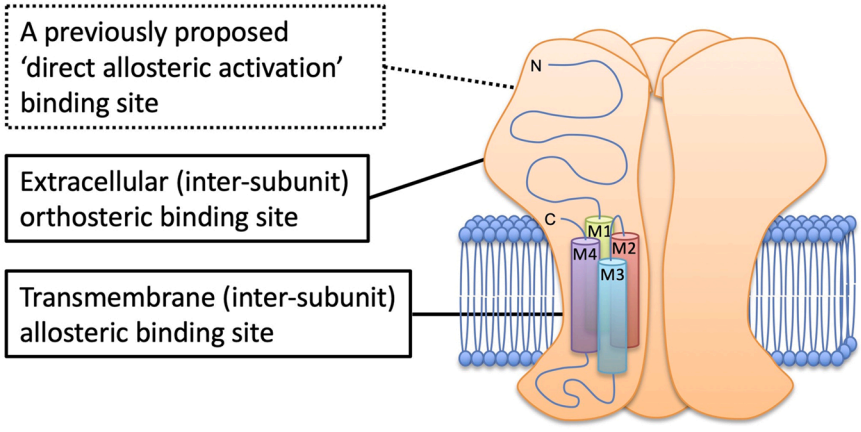

Fig. 1. Schematic representation of a homo-pentameric α7 nAChR. Evidence from a variety of sources (including high-resolution structures containing bound orthosteric and allosteric ligands) supports the conclusion that orthosteric ligands bind to an inter-subunit extracellular site and that at least some α7-selective allosteric modulators bind to a transmembrane inter-subunit site. In addition, it has been proposed previously that activation by allosteric agonists might require binding to both a transmembrane site and also a distinct 'direct allosteric activation site' located in the extracellular domain [40, 64].
Figure modified from Chatzidaki and Millar (2015) [12].

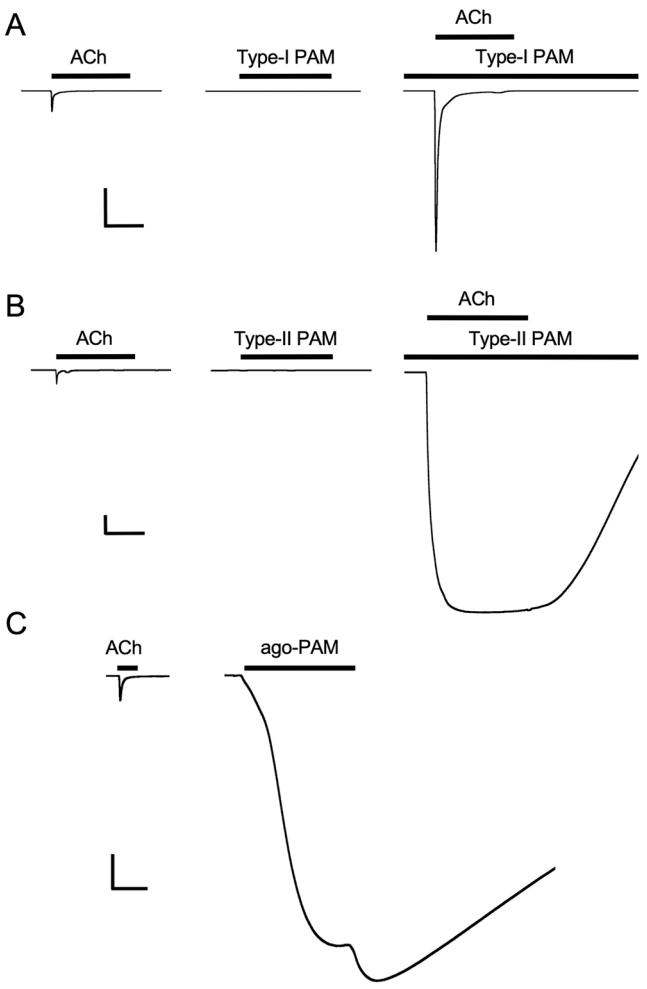

Fig. 2. Representative traces illustrating the effects of a type-I PAMs, type-II PAMs and ago-PAMs on α7 nAChRs. A) Type-I PAMs potentiate responses to ACh but have little or no effect on the rate of desensitisation. B) Type-II PAMs potentiate responses to ACh and cause significantly reduce the rate of agonist-evoked desensitisation. C) Ago-PAMs are able to activate α7 nAChRs in the absence of an orthosteric agonist and typically display a slower activation rage and induce considerably less desensitisation that orthosteric agonists. Horizontal scale bars are 5 s. Vertical scale bars are 0.2 μA (A and B) and 1 μA (C).

targets for therapeutic intervention in a range of neurological disorders. In addition to work conducted in academic research groups, this has also included many compounds that have been identified by pharmaceutical companies. Selected examples of novel α7 PAMs that were first described by pharmaceutical companies (many of which emerged from high-throughput screening approaches) include: PNU-120596 (identified by Pfizer) [20], LY 2807101 (Eli Lilly) [27], NS1738 (NeuroSearch/Abbott) [28], TQS (Abbott) [19], SB-206553 (Wyeth/Siena Biotech) [21], A-867744 (Abbott) [29], JNJ-1930942 (Johnson & Johnson) [22], Lu AF58801 (Lundbeck) [30], RO5126946 (Roche) [31], BNC375 (Bionomics/Prestwick Chemicals) [32] Compound 65 (Gedeon Richter) [33] and LL-00066471 (Lupin) [34]. In addition, numerous additional α7 nAChR PAMs have been described in patent applications [35] and also by academic research groups. This selective list of PAMs that were identified by pharmaceutical companies helps to illustrate the widespread interest in the possibility that PAMs acting on α7 nAChRs might have useful therapeutic applications [6,36]. Frustratingly, however, whilst several of these compounds have shown promise in pre-clinical studies, none as yet have progressed from clinical trials into therapeutic use. Somewhat ironically, galantamine (an acetylcholinesterase inhibitor that is used in the treatment of Alzheimer's disease), which is the only compound that is currently in clinical use that has been suggested to have α7 PAM activity [37], has subsequently been reported to lack any clear reproducible potentiating effect on α7 nAChRs [38]. As was mentioned previously, in humans (but not in other species) the α7 subunit can co-assemble with dupα7, a truncated α7 subunit that lacks the extracellular agonist-binding domain [8,9]. It is of interest that co-assembled α7/dupα7 complexes retain the ability to be modulated by α7-selective PAMs [9,39,40]. However, the existence dupα7 in humans (but not in animal models that are used widely in drug discovery) has been suggested as a possible factor contributing to the development of therapeutic drugs targeting α7nAChRs.

As will be discussed in more detail later, strong evidence has accumulated to suggest that many of these α7-selective PAMs bind to an allosteric site located in the nAChR transmembrane domain.

2.2. *Allosteric agonists ('ago-PAMs')*

Following the discovery of novel α7-selective PAMs, Millar and co-workers reported in 2011 that α7 nAChRs could be activated by an allosteric agonist (4BP-TQS) that acted through a site other than the conventional orthosteric site (Fig. 2) [41]. As will be discussed in more detail later, evidence has been obtained that suggests that direct allosteric activation of α7 nAChRs by compounds such as 4BP-TQS occurs by it binding to a site in the transmembrane domain [17,41–44]. Note: for a discussion of alternative nomenclature of 4BP-TQS and of other α7-selective allosteric modulators, see Section 2.4.

The starting point for the identification of α7-selective allosteric agonists was the knowledge that α7 nAChRs could be potentiated by PAMs such as TQS [19] (Table 1, Fig. 3). It was found that replacement of the naphthalene group of TQS with a 4-bromo-phenyl group (to create 4BP-TQS) resulted in a compound that displayed potent agonist activity on α7 nAChRs in the absence of an orthosteric agonist [41]. In contrast to the very rapid desensitisation of α7 nAChRs when activated by ACh, activation by 4BP-TQS causes little or no desensitisation [41]. In addition, in comparison to the endogenous agonist ACh, 4BP-TQS was found to have significantly higher efficacy and potency than ACh on α7 nAChRs [41]. The discovery in 2011 of 4BP-TQS [41] formed the bases for the synthesis and characterisation of an extensive series of TQS-related allosteric agonists that have been described by Millar and co-workers [17,42] and Papke and co-workers [45,46] (see Table 1, Fig. 3). More recently, an allosteric agonist B-973 has been identified that is chemically unrelated to the TQS-series of allosteric modulators [47]. As is the case with all of the TQS-series of allosteric agonists, B-973 also acts as a type-II PAM α7 nAChRs, as illustrated by its ability to potentiate responses to ACh [47,48].

2.3. *Diversity of allosteric modulators*

An interesting finding to emerge from studies with the TQS-series of compounds has been that only minor changes in chemical structure can dramatically alter their pharmacological effects as allosteric modulators of α7 nAChRs [17,41]. Indeed, this can occur as a result of altering just a single atom attached to a phenyl ring [42]. For example, replacing the bromine atom of 4BP-TQS with fluorine (a smaller halogen atom) to create 4FP-TQS results in the loss of allosteric agonist activity but the retention of PAM effects [42]. Similarly, allosteric agonist activity is lost (but PAM activity is retained) when a bromine atom is attached to either the *ortho* or *meta* position on the phenyl ring (2BP-TQS and 3BP-TQS), rather than to the *para* position (as in 4BP-TQS) [42]. These findings illustrate that both the position and the size of groups attached to a phenyl ring can be critical in determining allosteric agonist activity. Further evidence of how relatively small changes in chemical structure can have profound effects on allosteric modulation has been obtained from a series of nineteen compounds in which all combinations of methyl substitution of a single aromatic ring were examined [17]. Most of the nineteen methyl-substituted TQS compounds were found to be

Table 1
Nomenclature and chemical names of TQS-related α7 nAChR allosteric modulators.

Common name [REF]	Alternative names [REF]	Chemical name
2,3MP-TQS [17]		*cis-cis*-4-(2,3-Dimethylphenyl)– 3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulfonamide
2,3,4MP-TQS [17]		*cis-cis*-– 4-(2,3,4-Trimethylphenyl)– 3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*] quinoline-8-sulfonamide
2,3,4,5MP-TQS[17]		*cis-cis*-4-(2,3,4,5-Tetramethylphenyl)– 3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*] quinoline-8-sulfonamide
2,3,4,6MP-TQS[17]		*cis-trans*-4-(2,3,4,6-Tetramethylphenyl)– 3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*] quinoline-8-sulfonamide
2,3,5MP-TQS [17]		*cis-cis*-4-(2,3,5-Trimethylphenyl)– 3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*] quinoline-8-sulfonamide
2,3,5,6MP-TQS[17]	2,3,5,6TMP-TQS [50]; TMP-TQS[51]	*cis-trans*-4-(2,3,5,6-Tetramethylphenyl)– 3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*] quinoline-8-sulfonamide
2,3,6MP-TQS [17]		*cis-trans*-4-(2,3,6-Trimethylphenyl)– 3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*] quinoline-8-sulfonamide
2,4,5MP-TQS [17]		*cis-cis*-4-(2,4,5-Trimethylphenyl)– 3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*] quinoline-8-sulfonamide
2,4,6MP-TQS [17]		*cis-trans*-4-Mesityl-3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulfonamide
2,4MP-TQS [17]		*cis-cis*-4-(2,4-Dimethylphenyl)– 3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulfonamide
2,5MP-TQS [17]		*cis-cis*-4-(2,5-Dimethylphenyl)– 3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulfonamide
2,6MP-TQS [17]		*cis-trans*-4-(2,6-Dimethylphenyl)– 3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*] quinoline-8-sulfonamide
2BP-TQS[42]		*cis-cis*-4-(2-bromophenyl)– 3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulphonamide
2MP-TQS[17]		*cis-cis*-4-o-Tolyl-3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulfonamide
2 N-TQS[42]		*cis-cis*-4-(naphthalen-2-yl)– 3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulphonamide
3,4,5MP-TQS [17]		*cis-cis*-4-(3,4,5-trimethylphenyl)– 3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*] quinoline-8-sulfonamide
3,4BP-TQS [42]		*cis-cis*-4-(3,4-dibromophenyl)– 3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulphonamide
3,4MP-TQS [17]		*cis-cis*-4-(3,4-Dimethylphenyl)– 3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulfonamide
3,5MP-TQS [17]		*cis-cis*-4-(3,5-Dimethylphenyl)– 3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulfonamide
3BP-TQS[42]		*cis-cis*-4-(3-bromophenyl)– 3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulphonamide
3I-TQS[42]		*cis-cis*-4-(3-iodophenyl)– 3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulphonamide
3MP-TQS[17]		*cis-cis*-4-m-Tolyl-3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulfonamide
4BP-TQS[41]	GAT107 *[49]; GAT106 *[46]	*cis-cis*-4-(4-bromophenyl)– 3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulphonamide
4CP-TQS[42]		*cis-cis*-4-(4-chloroophenyl)– 3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulphonamide
4FP-TQS[42]		*cis-cis*-4-(4-fluorophenyl)– 3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulphonamide
4HP-TQS[42]		*cis-cis*-4-(4-hydroxyphenyl)– 3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulphonamide
4IP-TQS[42]		*cis-cis*-4-(4-iodoophenyl)– 3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulphonamide
4MP-TQS[42]		*cis-cis*-4-p-tolyl-3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulphonamide
GAT154[45]		*cis-cis*-4-(5-Bromothiophen-2-yl)– 3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*] quinoline-8-sulfonamide
GAT155[45]		*cis-cis*-4-(4-Bromothiophen-2-yl)– 3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*] quinoline-8-sulfonamide
GAT193[45]		*cis-cis*-4-(4-(3-Bromopropyl)phenyl)– 3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*] quinoline-8-sulfonamide
GAT904[45]		*cis-cis*-4-(4-(Bromomethyl)phenyl)– 3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*] quinoline-8-sulfonamide
GAT927[46]		*cis-cis*-4-(4,5,6,7-tetrahydrobenzo[*b*] thiophen-3-yl)– 3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulfonamide
PentaMP-TQS [17]		*cis-trans*-4-(2,3,4,5,6-Pentamethylphenyl)– 3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulfonamide
P-TQS[42]		*cis-cis*-4-phenyl-3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulphonamide
TQS[19]		*cis-cis*-4-(napthalen-1-yl)– 3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulphonamide

* Note: the name 'GAT106' has been used for the inactive (-)-enantiomer of 4BP-TQS and 'GAT107' for the active (+)-enantiomer.

either type-II PAMs or allosteric agonists (ago-PAMs), the exceptions being those that contain two *ortho* methyl groups (at positions 2 and 5 of the phenyl ring) and which exhibited effects typical of type-I PAMs, NAMs and SAMs [17].

2.4. Stereoisomers, enantiomers and nomenclature

A feature of several α7-selective allosteric modulators is that they can exist as different stereoisomers. In the case of the TQS-series of compounds it appears to be only the *cis-cis* diastereoisomers that act as allosteric agonists (ago-PAMs) [17,42,49]. In contrast, type-I PAM and NAM activity is observed with TQS compounds that preferentially form *cis-trans* diastereoisomers [17]. Individual stereoisomers can also exist as mirror images, referred to as (+) and (−) enantiomers, but are typically synthesised as racemic (±) mixtures. Recent studies have separated the two enantiomers of 4BP-TQS and have examined separately the (+) and (−) enantiomers [49]. It was found that only the (+) enantiomers was active [49], which would suggest that the purified (+) enantiomer and the racemic (±) mixture of 4BP-TQS would have essentially identical properties. When the two (+) enantiomers of 4BP-TQS were described by Thakur and co-workers, they were given new names. The active enantiomer (+)– 4BP-TQS has been named GAT107 [49] and the inactive enantiomer (−)– 4BP-TQS has been named GAT106 [46]. What is perhaps unfortunate is that the name GAT107 is now occasionally used in the literature without it being clear that this is an enantiomer of a what has been referred to frequently in the prior literature as 4BP-TQS. Another compound in the TQS-series of α7-selective allosteric modulators that has subsequently been renamed is 2,3,5,6MP-TQS (a name that was intended to denote the presence of methyl groups at the 2, 3, 5 and 6 positions of the phenyl ring) [17]. When this compound was subsequently examined it was renamed with the addition of an additional 'T' as 2,3,5,6TMP-TQS (although, this may

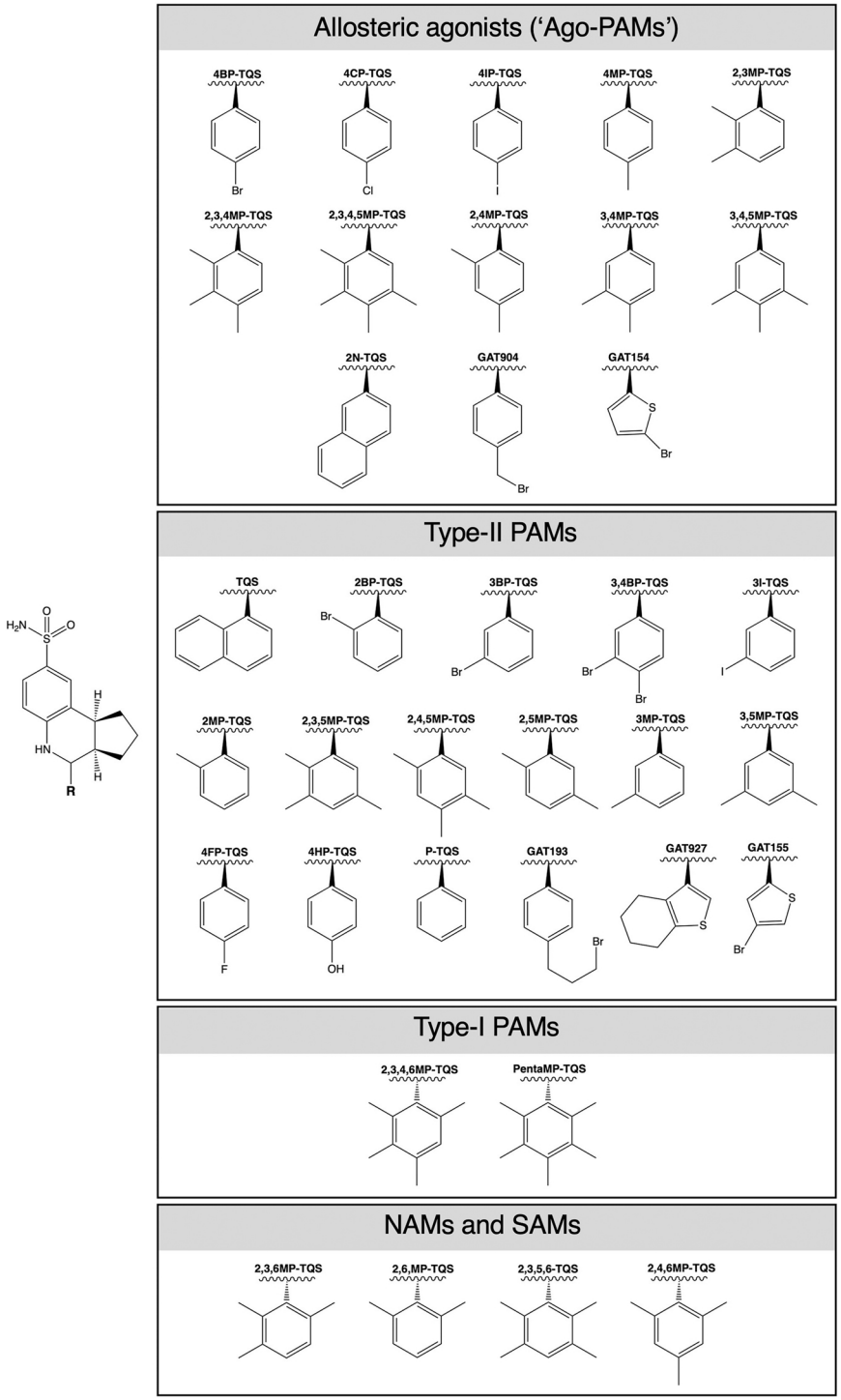

Fig. 3. Chemical structures of the 'TQS-series' of α7-selective nAChR allosteric modulators. As is illustrated on the left, all compounds in this series contain a quinoline ring attached to cyclopentane and sulfonamide groups, together with a variable aromatic group (R). Compounds isolated as either *cis-cis* diastereoisomers or *cis-trans* diastereoisomers are indicated by a solid or a hatched wedge-shaped bond at the R position, respectively. Although the distinction between type-I and type II PAMs is an oversimplification (as discussed in the text), for simplicity the compounds have been grouped into those displaying effects typical of allosteric agonists, type-I PAMs, type-II PAMs, NAMs and SAMs.

have been an inadvertent error) [50]. Subsequently, this new name has been abbreviated to 'TMP-TQS' [6,51], perhaps because 'TMP' was seen as a simpler way of denoting the presence of four (or 'tetra') methyl groups on the phenyl ring. However, this may have the potential to cause confusion since there were two separate compounds described in the original study of 'methyl-TQS' α7 modulators that contained four methyl groups but which exhibit different allosteric properties (2,3,4,5MP-TQS and 2,3,5,6MP-TQS) [17]. Of course, it is not uncommon for compounds used in pharmacological studies to be referred to by different names but it is something that can potentially cause confusion. For example, one α7-selective PAM has been referred to in scientific publications by at least four different names (for example, AVL-3288 [52], CCMI [53], XY-4083 [54] and Compound 6 [55]).

3. Binding sites for α7-selective allosteric modulators

3.1. A transmembrane binding site for α7-selective type-II PAMs

PNU-120596 was identified in 2005 as an α7-selective PAM that caused a dramatic loss of agonist-induced desensitisation [20] and it was the first example of what has been described as an α7-selective 'type-II' PAM. Subsequently, in 2008, further functional characterisation of

PNU-120596 involving the use of α7 subunit chimeras was conducted in two independent laboratories and led to the suggestion that the PAM effects of PNU-120596 were mediated via a transmembrane binding site [56,57]. Initially, a transmembrane site was proposed at an intra-subunit location [57]. This hypothesis was based, in part, on molecular docking studies that had been conducted with an α7 homology model that had originally been developed in 2006 by Changeux and co-workers [58] and which was based on the structure of a nAChR from the marine ray *Torpedo marmorata* (PDB 2BG9) [59]. However, when higher resolution structures of pLGICs were obtained, it became clear that a region of amino acids in the transmembrane region of the Torpedo nAChR structure had been incorrectly assigned to the electron density data in the original 2BG9 structure. This, in turn, suggested that the α7 homology model constructed in 2006 [58] was also likely to be incorrect. In 2017, a new α7 homology model was constructed by Millar and co-workers [43]. Once again, it was based on the Torpedo nAChR structure but, importantly, after correcting the presumed error in the transmembrane domain [43]. Molecular docking studies conducted with this revised α7 homology model led to the proposal that α7-selective type-II PAMs such as PNU-120596 bound to a transmembrane site at the interface of adjacent subunits (an inter-subunit site) [43], rather than at an intra-subunit transmembrane site, as had been proposed previously.

Recently (in 2021) two independent groups succeeded in generating high-resolution structures of the human α7 nAChRs [12,13] by means of cryogenic electron microscopy (cryo-EM). Furthermore, both groups obtained structures of α7 in complex with the type-II PAM, PNU-120596 (PDB 7KOX and 7EKT) [12,13]. Whilst it was not possible to determine the position of the bound PNU-120596 in one of these structures (7KOX) [12], the position of PNU-120596 in 7EKT was clearly identified and was located at an inter-subunit site in the transmembrane domain [13]. Remarkably, the position of the bound PAM is in very close agreement with the position that had been predicted by molecular docking studies in the revised (2017) α7 homology model [43]. In addition, PNU-120596 was found to interact in the cryo-EM structure with a methionine residue at the 15' position of the second transmembrane helix (TM2) that had been shown previously by site-directed mutagenesis to be a critical determinant of potentiation by both PNU-120596 and other chemically diverse α7-selective allosteric modulators [17,41–43,57,60,61].

This would seem to provide overwhelming evidence that type-II PAMs such as PNU-120596 bind to an inter-subunit cavity in the α7 transmembrane domain. A mechanism has been proposed, whereby the binding of a PAM (in the absence of an orthosteric agonist) is insufficient to stabilise the open conformation of the receptor but when an agonist is bound to the orthosteric site, the combined effect of the two ligands is to stabilise the receptor in a non-desensitised open state. In addition, a considerable amount of experimental data has been obtained that is consistent with this being the binding site for all currently identified α7-selective type-II PAMs. This includes data derived from studies with subunit chimeras, site-directed mutagenesis, radioligand binding and molecular docking. It also includes studies conducted with a range of type-II PAMs, including A-867744, LY-2087101, PNU-120596, TBS-516 and TQS [17,42,43,56,57,60–63].

On the basis of early studies with subunit chimeras it was suggested that the nAChR extracellular domain played a role in allosteric modulation by type-I PAMs such as NS1738 [56], whereas there is pharmacological data supporting the proposal that type-I and -II PAMs can act competitively, as might be expected if they shared an overlapping or mutually-exclusive transmembrane binding site [61]. It seems plausible that the allosteric effects of both type-I and type -II PAMs (and also PAMs described as having intermediate effects) might all be mediated via a common transmembrane site, although the possibility that they act through different sites is also possible.

3.2. A transmembrane binding site for allosteric agonists (ago-PAMs)

As was discussed in the previous section, following the recent determination of the cryo-EM structure of an α7 nAChR bound to PNU-120596 [13], there seems little doubt that α7-selective type-II PAMs exert their potentiating effects by binding to an inter-subunit transmembrane site. In addition, there is considerable amount of experimental evidence to suggest that this is also the site at which α7-selective ago-PAMs such as 4BP-TQS act as allosteric agonists. This would also be consistent with evidence that allosteric agonists of other pLGIC act via transmembrane binding sites [64–66]. However, since the precise location of the binding site for α7-selective ago-PAMs is currently a somewhat contentious issue, it will be reviewed in more detail here.

Initial evidence that compounds such as 4BP-TQS activate α7 nAChRs via a different mechanism from that of orthosteric agonists was provided by its much slower activation rate than ACh [41], its ability to activate α7 nAChRs without causing rapid desensitisation [41] and the differences in single channel kinetics when α7 nAChRs are activated by 4BP-TQS, rather than by ACh [67]. In addition, there is evidence that activation of α7 nAChRs by ACh and 4BP-TQS display differing temperature sensitivity [68]. Further evidence that compounds such as 4BP-TQS do not exert their agonist effects via the conventional extracellular orthosteric site came from studies conducted with antagonists such as methyllycaconitine (MLA). MLA is as a competitive antagonist of responses to ACh, as is illustrated by its antagonist effects being surmountable at high agonist concentrations. However, MLA causes non-surmountable antagonism of responses to 4BP-TQS [41]. In addition, competition radioligand binding experiments have shown that α7 allosteric agonists do not displace the binding of radioligands to the orthosteric site of α7 nAChRs [17].

Some of the most direct experimental evidence suggesting that α7-selective allosteric agonists act at a transmembrane site comes from studies with α7 subunit chimeras. For example, allosteric agonists such as 4BP-TQS display no agonist activity on subunit chimeras containing the α7 extracellular domain fused to the transmembrane domain from a pLGIC subunit that is insensitive to such compounds (Fig. 4) [41]. Conversely, allosteric agonist-activity is retained in subunit chimeras that contain the α7 transmembrane domain fused to the extracellular domain of a pLGIC subunit that is insensitive to such compounds (Fig. 4) [69].

In addition, there are several examples where site-directed mutagenesis of individual amino acids has resulted in dramatically differing effects on activation by orthosteric and allosteric agonists. For example, mutation of a single tyrosine residue that is close to the extracellular orthosteric binding site of the α7 nAChRs abolishes activation by orthosteric agonists but has little or no effect on direct activation by allosteric agonists (Fig. 4) [70]. Similarly, mutation of the 15' position in the TM2 helix of the α7 transmembrane domain (which is an amino acid that PNU-120596 was found to interact with in the α7 cryo-EM structure 7EKT [13]) has dramatic effects on responses to allosteric agonists but not orthosteric agonists. When the methionine at this position in α7 is mutated to leucine (M253L) it has no significant effect on agonist responses to orthosteric agonists but completely abolishes direct allosteric activation by ago-PAMs (Fig. 4) [17,41,42]. It is a remarkably dramatic effect and one that has been observed with many different α7-selective ago-PAMs, including 4BP-TQS [17,41,42]. Interestingly, whereas α7-selective PAMs such as TQS have no allosteric modulatory effects on heteromeric nAChRs such as α4β2, mutations at the same (15') position of the TM2 helix of the β2 subunit enables α7-selective PAMs such as TQS to act as allosteric agonists on α4β2 nAChRs [46].

In addition, molecular docking studies have provided further support for α7-selective allosteric agonists such as 4BP-TQS bind to a transmembrane site. For example, when docking studies were performed with 25 different α7-selective allosteric modulators from the TQS-series of compounds (11 ago-PAMs and 14 type-II PAMs), all 25 compounds docked preferentially in overlapping positions and in the same

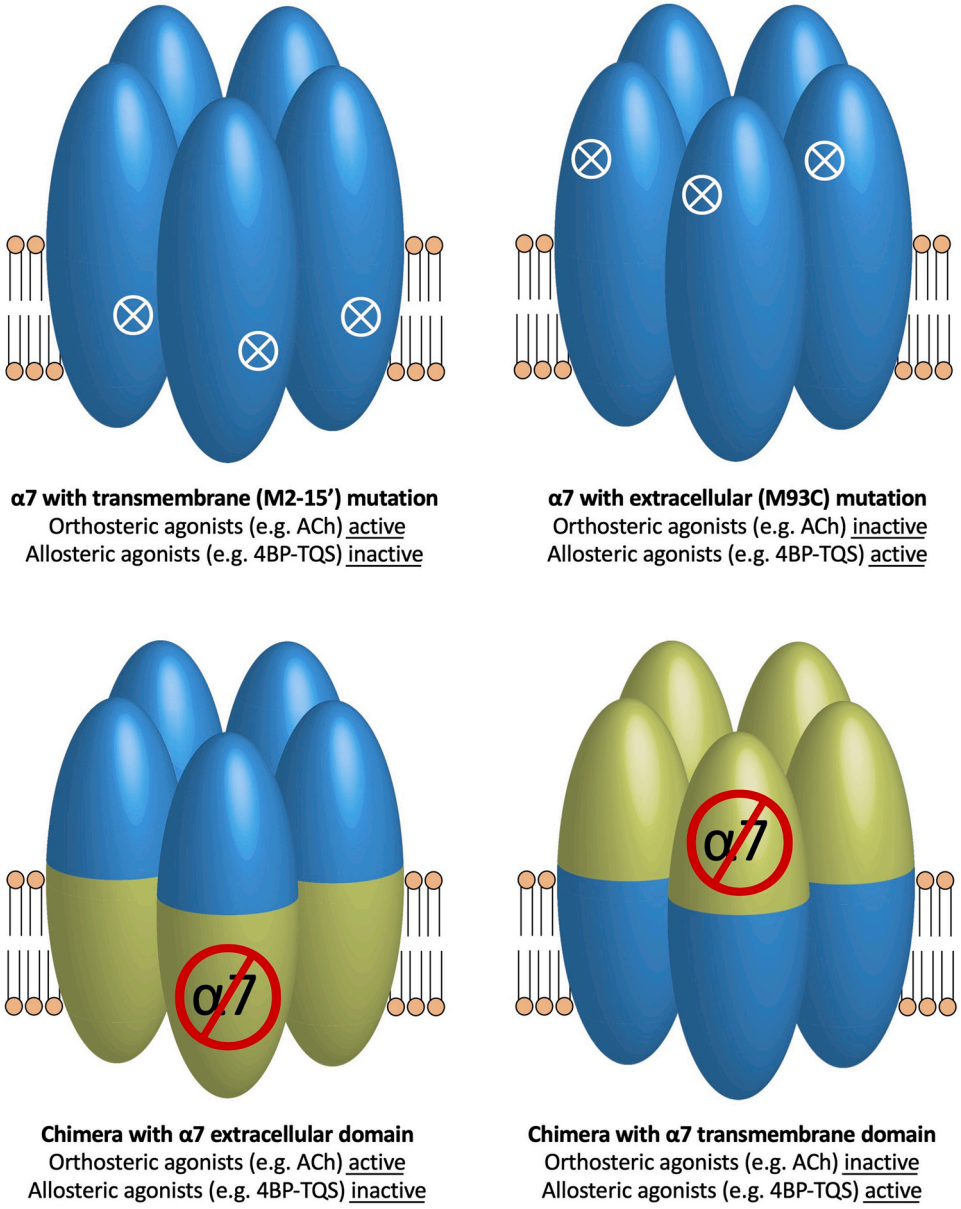

Fig. 4. Studies conducted with subunit chimeras and by site-directed mutagenesis. Whilst it is possible that both site-directed point mutation and subunit chimeras may cause long-range conformational changes, the results of several such studies (as discussed in the text) are consistent with the hypothesis that α7-selective ago-PAMs such as 4BP-TQS act as allosteric agonists by binding to a transmembrane site.

orientation at an α7 inter-subunit transmembrane site [43]. Significantly, this predicted binding site for both α7-selective type-II PAMs and ago-PAMs is in very good agreement with the recently identified transmembrane binding site for PNU-120596 in the α7 nAChR cryo-EM structure (7EKT) [13].

An important contribution to our understanding of how compounds in the TQS series may interact with the α7 nAChR has come from the separation of their (+)- and (−)-enantiomers. Whilst it was known that both type-II PAMs and ago-PAMs in the TQS series were *cis-cis*-diastereoisomers [17,42], it has also been shown that only one of the two (+)/(−) enantiomers (which are mirror-images of one another) are active. For example, it has been found that the active enantiomer of 4BP-TQS (an ago-PAM) is its (+)-enantiomer [49], whereas the active enantiomer of TQS (a type-II PAM) is its (−)-enantiomer [46]. Importantly, however, it turns out that the active forms of these two compounds ((+)− 4BP-TQS and (−)-TQS) have the same stereochemistry (3aR,4 S,9bS) and hence the same relative geometric orientation. This is a finding that would certainly be consistent with the two classes of allosteric modulator (type-II PAM and ago-PAM) interacting with a common binding site, albeit, presumably differing in their ability to stabilise an open channel conformation in the absence of an orthosteric agonist.

3.3. Is a second (extracellular) site required to explain direct allosteric activation?

Whilst there appears to be a general consensus that both PAMs (such as TQS) and ago-PAMs (such as 4BP-TQS) bind to a transmembrane 'PAM site', it has been suggested that, in order to exert their allosteric agonist effects, ago-PAMs also need to bind to a second independent site in the extracellular domain, referred to as the 'allosteric direct activation binding site' [70]. Whereas this two-site hypothesis may have initially been proposed as being just one possible explanation for the differences between PAMs (such as TQS) and ago-PAMs (such as 4BP-TQS) [70], in

recent years it has been presented as an almost settled argument. For example, it has been stated that direct allosteric activation by ago-PAMs 'involves binding to two distinct sites' (transmembrane and extracellular) [71] and that α7-selective allosteric agonists 'bind to an allosteric activation (AA) site in the extracellular domain' (in addition to the transmembrane PAM site) [72].

It seems that a primary motivation for proposing a second structurally-independent (extracellular) binding site for the direct agonist activation of α7 nAChRs was to explain a phenomenon that has been described as 'primed potentiation' [70]. This is an effect that is observed after the application of a high concentration of an allosteric agonist, in which long-lasting potentiation of responses to orthosteric agonists can be detected long after the allosteric agonist has been washed out of the recording chamber [45,70]. It is this phenomenon of primed potentiation that appears to have led to the initial suggestion that allosteric agonists might bind to both a transmembrane 'PAM site' (from which it is assumed to dissociate extremely slowly), and also to an extracellular 'direct activation site', from which it is assumed to dissociate much more rapidly.

There may however be an alternative explanation for what has been described as 'primed potentiation'. It is connected with the fact that the 'PAM effects' of ago-PAMs such as 4BP-TQS can be detected at very much lower concentrations than are required for direct allosteric activation. A common observation with compounds such as 4BP-TQS is that they appear to stick non-specifically but quite persistently to some surfaces, such as those that can be found in typical electrophysiological recording systems (for example, plastic tubing used for perfusion and/or recording chambers). It may also be possible for compounds to adhere non-specifically (or to partition into) and then subsequently dissociate from the large oocyte cells that are frequently used for functional characterisation of α7-selective ago-PAMs. It seems possible that the subsequent slow dissociation of low concentrations of ago-PAMs from such surfaces (and subsequent re-association with the α7 nAChR) could explain what has been described as primed potentiation. If such an explanation was correct, there might be no need to postulate two structurally and topologically distinct selective binding sites for ago-PAMs on α7 nAChRs (at least as an explanation for primed potentiation). However, the phenomenon of primed potentiation is certainly intriguing and it is something that is likely to require further study before we fully understand its mechanism of action. It is undoubtedly possible that it has a more interesting and unexpected pharmacological basis than being due to compounds sticking to (and then slowly dissociating from) plasticware or oocyte compartments.

3.4. Why is the second (proposed) ago-PAM site assumed to be extracellular?

There is certainly a comforting orthodoxy about the idea that allosteric agonists bind to the extracellular domain of nAChRs, since this is where we know it is possible for orthosteric agonists such as ACh to facilitate receptor activation. However, if it is necessary to propose a secondary independent binding site to explain effects such as primed potentiation, a separate question is: why would it be assumed to be located at the extracellular domain? An argument that has been put forward is that allosteric agonists such as 4BP-TQS dissociate rapidly from the 'direct activation site' (which might imply a lower affinity site) but do so more slowly from the transmembrane 'PAM site' [70]. The rationale appears to be that rapid dissociation would be more likely from an extracellular site than a transmembrane site. Originally, a model was suggested in which the proposed 'allosteric direct activation site' was below the orthosteric binding site (i.e., closer to the transmembrane domain than the orthosteric site) [70]. It was an idea that was based on docking studies with an α7 homology model (although the data was not presented) [70]. Subsequently, an alternative extracellular location was proposed that was situated above the orthosteric binding site (i.e., further away from the transmembrane domain) and this has been referred to as the 'vestibular site' [45]. This modified hypothesis was also based on molecular docking studies which were conducted with a homology model containing only the extracellular domain α7 receptor [45]. The homology model of the α7 extracellular domain was based upon a modified version of an acetylcholine binding protein (AChBP) from the snail *Lymnaea stagnalis* (a soluble protein that is considered to be a structural homologue of the nAChR extracellular domain) [73,74]. Two putative extracellular binding sites were identified and, based on a variety of arguments, the authors explained why they favoured the vestibule binding site as the probable site of direct agonist activation [45]. In subsequent molecular docking studies conducted by the same group (again, conducted with a homology model corresponding to only the extracellular domain), docked positions of allosteric agonists were identified in multiple extracellular sites (including the orthosteric site as well as the proposed vestibule site) [75]. It was argued that docked positions that were shared by an allosteric agonist (e.g., 4BP-TQS) and by a PAM that lacked agonist effects (e.g., TQS) would be unlikely to be responsible for direct allosteric activation. For that reason, docking studies with these two allosteric modulators were compared and it was reported that the two ligands did, indeed, bind in opposing orientations in the proposed vestibule site [75]. However, it appears that, whereas docking was performed with the active enantiomer of 4BP-TQS (which, as discussed earlier, is 3aR,4 S,9bS), these docking results were compared to an enantiomer of TQS (3aS,4 R,9bR) that has been shown to be inactive [46]. Becasue the stereochemical relationship of the two compounds used for these docking studies is essentially that of a mirror image, the fact that a site was identified at which they were predicted to bind in opposing orientations would not appear to be a plausible reason for selecting it as a likely site for direct allosteric activation.

There is no reason, in principle, why an allosteric modulatory site should not be located in the extracellular domain. Indeed, there is evidence that some compounds that inhibit ACh-induced responses in α7 nAChRs are able to bind to a soluble AChBP that has been modified to have greater sequence similarity to the α7 nAChR extracellular domain [76,77]. Similarly, it has also been reported that proteins that can bind to the extracellular domain of pLGICs (such as single domain antibodies) can also cause either PAM or NAM effects [78,79]. However, the hypothesis that direct allosteric agonist activation of α7 nAChR requires the simultaneous binding of a single ligand to two separate and distinct binding sites (transmembrane and extracellular) could be seen as being an unusual pharmacological mechanism of action. It is a hypothesis that would appear to require two structurally and topographically distinct binding sites on the α7 nAChR to have evolved independently in such a way that both sites were able to confer selective and specific binding for structurally dissimilar allosteric agonists (such as 4BP-TQS and B-973).

A potential source of confusion when discussing this topic is that the term 'allosteric activation' has also been used to describe activation of α7 nAChRs by compounds such as the α-conotoxin MrIC that have no intrinsic agonist effect when applied by themselves but which can act as agonists when co-applied with PAMs and which have been proposed to act via the postulated vestibular 'direct allosteric direct activation site' [51]. α-Conotoxins are small (12–10 amino acid) peptides that typically act as competitive antagonists of nAChRs by binding to the orthosteric site [80] however MrIC has been shown to act as an agonist of α7 nAChRs when co-applied with PNU-120596 [81]. Due to its agonist effects being observed only with PAMs, MrIC has been described as a 'biased agonist' by the group that originally characterised its effects [82], an effect that was presumably assumed to be mediated by MrIC binding to the same orthosteric site at which α-conotoxins act as competitive antagonists. However, proponents of the 'direct allosteric activation site' hypothesis, have also argued that MrIC may cause allosteric activation via the same proposed vestibular site. Indeed, by performing docking studies that were restricted to just the proposed vestibular site, a potential binding site for MrIC was suggested in the proposed vestibular site [51].

3.5. An alternative single-site hypothesis of cooperative allosteric interactions

In contrast to the hypothesis that was discussed in the preceding section concerning the possibility of there being two distinct (transmembrane and extracellular) binding sites for allosteric agonists, there may be a simpler explanation that could account for all of the experimental observations. It involves the idea that the pharmacological effects of ligands binding to multi-subunit proteins such as nAChRs can be influenced by the number of binding sites that are occupied. Even for a 'single' transmembrane binding site model, there will be five such sites in a homo-pentameric receptor that are potentially able to cause induced conformational changes in neighbouring sites within the same oligomeric complex. This idea is consistent with the well-established phenomenon of 'cooperative binding' of ligands to sites on multi-subunit proteins. It is possible that, if an allosteric modulator binds with low occupancy to a subset of the five α7 inter-subunit transmembrane sites, it might induce a conformational change in neighbouring subunits within the same oligomer that could alter the kinetics or the selectivity of ligand binding to those neighbouring sites.

As has been reviewed elsewhere, cooperative binding (both positive and negative) of ligands to multi-subunit proteins is a mechanism that has been shown to underly a wide range of biological processes [83]. The cooperative interaction of binding sites is an idea that was proposed in 1904 as means of explaining the interaction of oxygen with haemoglobin [84] and has been developed into what is often described as the 'Monod-Wyman-Changeux' model of concerted allosteric transitions [83,85]. It is a mechanism in which ligand occupancy of binding sites in a multi-subunit protein is not a linear function of ligand concentration and, depending on the nature of the conformational change that occurs in neighbouring subunits, the resulting cooperative effect can be either positive or negative. It seems that such an effect could explain all of the experimental data that has been obtained concerning allosteric modulation of nAChRs (including primed potentiation).

In fact, to explain several of the effects observed with α7 ago-PAMs does not necessarily even require ligand binding to be 'cooperative', since the observed effects may depend simply on a cumulative effect, reflecting how many of the five equivalent transmembrane sites are occupied. For example, the observation that ago-PAMs require higher concentrations for direct allosteric activation than are required to exert PAM effects may simply be a consequence of direct allosteric activation requiring higher ligand occupancy at the transmembrane sites in order to stabilise an open conformation. Direct allosteric activation of α7 nAChRs by compounds such as 4BP-TQS has much slower activation kinetics than is seen with orthosteric agonists [41] and this could perhaps be explained by direct allosteric activation requiring higher receptor occupancy by an allosteric agonist at a relatively inaccessible transmembrane site. This is in contrast to the rapid activation of α7 nAChRs by orthosteric agonists, for which it has been shown that efficient receptor activation can be achieved even with low occupancy of the five extracellular orthosteric agonist sites [14]. However, if a pharmacological explanation is needed to explain effects such as primed potentiation, this could be due to slower dissociation from the allosteric transmembrane sites when there is lower ligand occupancy of those sites. Such an effect would be consistent with existing ideas in which ligand binding can cause cooperative conformational changes in neighbouring subunits. Similarly, where an antagonist has been observed to have differing effects on direct allosteric activation than it does with either PAM effects or with 'primed potentiation', as has been reported with compounds such as 2,3,5,6MP-TQS [72], this could be due to cooperative conformational changes in the five transmembrane binding sites resulting from differing ligand occupancy.

4. Conclusion

With the recent determination of a cryo-EM structure of the α7 nAChR containing a bound PAM, together with a wealth of additional experimental data, it seems clear that α7-selective type-II PAMs such as PNU-120596 and TQS bind to an inter-subunit transmembrane binding site. There also appears to be a general consensus that allosteric agonists/ago-PAMs (such as 4BP-TQS) also bind to the same site. What remains unresolved is whether direct allosteric agonist activation also requires the simultaneous binding of compounds such as 4BP-TQS to an additional distinct extracellular site, rather than a single transmembrane site shared with type-II PAMs. A possible attraction of a mechanism in which direct allosteric activation involves a single set of cooperative (transmembrane) allosteric sites is its simplicity. In a homo-pentameric nAChR such as α7, it would involve the binding of allosteric modulators to a single set of five essentially equivalent transmembrane sites, rather than to two structurally and topologically distinct sets of binding sites (a set of five transmembrane sites and, additionally, a set of five extracellular sites). Of course, simplicity (which is often said to underlie the 'principle of parsimony', or 'Occam's razor') is not, in itself, a basis upon which to favour one scientific hypothesis over another. However, the idea of a single class of binding sites for ago-PAMs, located at the interface of adjacent subunits in the transmembrane domain (the same location where we know type-II PAMs bind) is a hypothesis that appears to fit all of the experimental data and is also consistent with well-established principles of cooperative binding that has been used to explain the pharmacological effects of ligand binding to numerous multi-subunit proteins.

Declaration of Competing Interest

None.

Data Availability

No new unpublished data are presented in this review article.

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References

[1] P.-J. Corringer, F. Poitevin, M.S. Prevost, L. Sauguet, M. Delarue, J.-P. Changeux, Structure and pharmacology of pentameric receptor channels: from bacteria to brain, Structure 20 (2012) 941–956.
[2] J.-P. Changeux, The nicotinic acetylcholine receptor: the founding father of the pentameric ligand-gated ion channel superfamily, J. Biol. Chem. 287 (2012) 40207–40215.
[3] N.S. Millar, A review of experimental techniques used for the heterologous expression of nicotinic acetylcholine receptors, Biochem Pharm. 78 (2009) 766–776.
[4] N.S. Millar, C. Gotti, Diversity of vertebrate nicotinic acetylcholine receptors, Neuropharmacol 56 (2009) 237–246.
[5] S. Couturier, D. Bertrand, J.M. Matter, M.C. Hernandez, S. Bertrand, N. Millar, et al., A neuronal nicotinic acetylcholine receptor subunit (α7) is developmentally regulated and forms a homo-oligomeric channel blocked by α-BTX, Neuron 5 (1990) 847–856.
[6] R.L. Papke, N.A. Horenstein, Therapeutic targetting of α7 nicotinic acetylcholine receptors, Pharm. Ther. 73 (2021) 1118–1149.
[7] S.S. Khiroug, P.C. Harkness, P.W. Lamb, S.N. Sudweeks, L. Khiroug, N.S. Millar, et al., Rat nicotinic receptor α7 and β2 subunits co-assemble to form functional heteromeric nicotinic receptor channels, J. Physiol. 540 (2002) 425–434.
[8] M.L. Sinkus, S. Graw, R. Freedman, R.G. Ross, H.A. Lester, S. Leonard, The human CHRNA7 and CHRFAM7A genes: A review of the genetics, regulation, and function, Neuropharmacology 96 (2015) 274–288.
[9] M. Lasala, J. Corradi, A. Bruzzone, Md.C. Esandi, C. Bouzat, A human-specific, truncated 7 nicotinic receptor subunit assembles with full-length 7 and forms functional receptors with different stoichiometries, J. Biol. Chem. 293 (2018) 10707–10717.
[10] R.M. Walsh, S.-H. Roh, A. Gharpure, C.L. Morales-Perez, J. Teng, R.E. Hibbs, Structural principles of distinct assemblies of the human α4β2 nicotinic receptor, Nature 557 (2018), 261-165.

[11] A. Gharpure, J. Teng, Y. Zhuang, C.M. Noviello, R.M. Walsh, R. Cabuco, et al., Agonist selectivity and ion permeation in the α3β4 ganglionic nicotinic receptor, Neuron 104 (2019) 501–511.

[12] C.M. Noviello, A. Gharpure, N. Mukhtasimova, R. Cabuco, L. Baxter, D. Borek, et al., Structure and gating mechanism of the α7 nicotinic acetylcholine receptor, Cell 184 (2021) 2121–2134.

[13] Y. Zhao, S. Liu, Y. Zhou, M. Zhang, H. Chen, H.E. Xu, et al., Structural basis of human α7 nicotinic acetylcholine receptor activation, Cell Res 31 (2021) 713–716.

[14] D. Rayes, M.J. De Rosa, S.M. Sine, C. Bouzat, Number and locations of agonist binding sites required to activate homomeric Cys-loop receptors, J. Neurosci. 29 (2009) 6022–6032.

[15] J. Wang, J. Lindstrom, Orthosteric and allosteric potentiation of heteromeric neuronal nicotinic acetylcholine receptors, Br. J. Pharm. (2017).

[16] A. Chatzidaki, N.S. Millar, Allosteric modulation of nicotinic acetylcholine receptors, Biochem Pharm. 97 (2015) 408–417.

[17] J.K. Gill-Thind, P. Dhankher, J.M. D'Oyley, T.D. Sheppard, N.S. Millar, Structurally similar allosteric modulators of α7 nicotinic acetylcholine receptors exhibit five distinct pharmacological effects, J. Biol. Chem. 290 (2015) 3552–3562.

[18] D. Bertrand, M. Gopalakrishnan, Allosteric modulation of nicotinic acetylcholine receptors, Biochem Pharm. 74 (2007) 1155–1163.

[19] J.H. Grønlien, M. Håkerud, H. Ween, K. Thorin-Hagene, C.A. Briggs, M. Gopalakrishnan, et al., Distinct profiles of α7 nAChR positive allosteric modulation revealed by structurally diverse chemotypes, Mol. Pharm. 72 (2007) 715–724.

[20] R.S. Hurst, M. Hajós, M. Raggenbass, T.M. Wall, N.R. Higdon, J.A. Lawson, et al., A novel positive allosteric modulator of the α7 neuronal nicotinic acetylcholine receptor: in vitro and in vivo characterization, J. Neurosci. 25 (2005) 4396–4405.

[21] J. Dunlop, T. Lock, B. Jow, F. Sitzia, S. Grauer, F. Jow, et al., Old and new pharmacology: positive allosteric modulation of the α7 nicotinic acetylcholine receptor by the 5-hydroxytryptamine$_{2B/C}$ receptor antagonist SB-206553 (3,5-dihydro-5-methyl-*N*-3-pyridinylbenzo[1,2-*b*:4,5-*b*']di pyrrole-1(2*H*)-carboxamide), J. Pharm. Exp. Ther. 328 (2009) 766–776.

[22] T. Dinklo, H. Shaban, J.W. Thuring, H. Lavreysen, K.E. Stevens, L. Zheng, et al., Characterization of 2-[[4-fluoro-3-(trifluoromethyl)phenyl]amino]-4-(4-pyridinyl)-5-thiazolethanol (JNJ-1930942), a novel positive allosteric modulator of the α_7 nicotinic acetylcholine receptor, J. Pharm. Exp. Ther. 336 (2011) 560–574.

[23] R. Zwart, G. De Filippi, L.M. Broad, G.I. McPhie, K.H. Pearson, T. Baldwinson, et al., 5-Hydroxyindole potentiates human α7 nicotinic receptor-mediated responses and enhances acetylcholine-induced glutamate release in cerebellar slices, Neuropharmacol 43 (2002) 374–384.

[24] R.M. Krause, B. Buisson, S. Bertrand, P.-J. Corringer, J.-L. Galzi, J.-P. Changeux, et al., Ivermectin: a positive allosteric effector of the α7 neuronal nicotinic acetylcholine receptor, Mol. Pharm. 53 (1998) 283–294.

[25] W.G. Conroy, Q.-S. Liu, Q. Nai, J.F. Margiotta, D.K. Berg, Potentiation of α7-containing nicotinic acetylcholine receptors by select albumins, Mol. Pharm. 63 (2003) 419–428.

[26] F. Chimienti, R.C. Hogg, L. Plantard, C. Lehmann, N. Brakch, J. Fischer, et al., Identification of SLURP-1 as epidermal neuromodulator explains the clinical phenotype of Mal de Meleda, Hum. Mol. Genet 12 (2003) 3017–3024.

[27] L.M. Broad, R. Zwart, K.H. Pearson, M. Lee, L. Wallace, G.I. McPhie, et al., Identification and pharmacological profile of a new class of selective nicotinic acetylcholine receptor potentiators, J. Pharm. Exp. Ther. 318 (2006) 1108–1117.

[28] D.B. Timmermann, J.H. Grønlien, K.L. Kohlhaas, E.Ø. Nielsen, E. Dam, T. D. Jørgensen, et al., An allosteric modulator of the α7 nicotinic acetylcholine receptor possessing cognition-enhancing properties in vivo, J. Pharm. Exp. Ther. 323 (2007) 294–307.

[29] R. Faghih, S.M. Gopalakrishnan, J.H. Gronlien, J. Malysz, C.A. Briggs, C. Wetterstrand, et al., Discovery of 4-(5-(4-chlorophenyl)-2-methyl-3-propionyl-1H-pyrrol-1-yl)benzenesulfonamide (A-867744) as a novel positive allosteric modulator of the α7 nicotinic acetylcholine receptor, J. Med Chem. 52 (2009) 3377–3384.

[30] J. Eskildsen, J.P. Redrobe, A.G. Sams, K. Dekermendjian, M. Laursen, J.B. Boll, et al., Discovery and optimization of Lu AF58801, a novel, selective and brain penetrant positive allosteric modulator of alpha-7 nicotinic acetylcholine receptors: attenuation of subchronic phencyclidine (PCP)-induced cognitive deficits in rats following oral administration, Bioorg. Med. Chem. Lett. 24 (2014) 288–293.

[31] S. Sahdeo, T. Wallace, R. Hirakawa, F. Knoflach, D. Bertrand, H. Maag, et al., Characterization of RO5126946, a novel α7 nicotinic acetylcholine receptor-positive allosteric modulator, J. Pharm. Exp. Ther. 350 (2014) 455–468.

[32] A.J. Harvey, T.D. Avery, L. Schaeffer, C. Joseph, B.C. Huff, R. Singh, et al., Discovery of BNC375, a potent, selective, and orally available type I positive allosteric modulator of α7 nAChRs, ACS Med Chem. Lett. 10 (2019) 754–760.

[33] I. Ledneczki, P. Tapolcsányi, E. Gábor, A. Visegrády, M. Vass, J. Éles, et al., Discovery of novel positive allosteric modulators of the α7 nicotinic acetylcholine receptor: Scaffold hopping approach, Eur. J. Med Chem. 214 (2021), 113189.

[34] M.K. Verma, R.N. Goel, A.M. Bokare, M.P. Dandekar, S. Koul, S. Desai, et al., LL-00066471, a novel positive allosteric modulator of α7 nicotinic acetylcholine receptor ameliorates cognitive and sensorimotor gating deficits in animal models: discovery and preclinical characterization, Eur. J. Pharm. (2021) 891.

[35] D. Gündisch, C. Eibl, Nicotinic acetylcholine receptor ligands, a patent review (2006-2011), Expert Opin. Ther. Pat. 21 (2011) 1867–1896.

[36] V.V. Uteshev, Allosteric modulation of nicotinic acetylcholine receptors: the concept and the therapeutic trends, Curr. Pharm. Des. 22 (2016) 1986–1997.

[37] M. Samochocki, A. Höffle, A. Fehrenbacher, R. Jostock, J. Ludwig, C. Vhristner, et al., Galantamine is an allosterically potentiating ligand of neuronal nicotinic but not of muscarinic acetylcholine receptors, J. Pharm. Exp. Ther. 305 (2003) 1024–1036.

[38] N.M. Kowal, P.K. Ahring, V.W.Y. Liao, D.C. Indurti, B.S. Harvey, S.M. O'Connor, et al., Galantamine is not a positive allosteric modulator of human α4β2 or α7 nicotinic acetylcholine receptors, Br. J. Pharm. 175 (2018) 2911–2925.

[39] I. Ihnatovych, T.K. Nayak, A. Ouf, N. Sule, B. Birkaya, L. Chaves, et al., iPSC model of CHRFAM7A effect on α7 nicotinic acetylcholine receptor function in the human context, Transl. Psychiatry 9 (2019) 59.

[40] C. Martín-Sánchez, E. Alés, S. Balseiro-Gómez, G. Atienza, F. Arnalich, A. Bordas, et al., The human-specific duplicated α7 gene inhibits the ancestral α7, negatively regulating nicotinic acetylcholine receptor-mediated transmitter release, J. Biol. Chem. 296 (2021), 100341.

[41] J.K. Gill, M. Savolainen, G.T. Young, R. Zwart, E. Sher, N.S. Millar, Agonist activation of α7 nicotinic acetylcholine receptors via an allosteric transmembrane site, Proc. Natl. Acad. Sci. USA 108 (2011) 5867–5872.

[42] J.K. Gill, P. Dhankher, T.D. Sheppard, E. Sher, N.S. Millar, A series of α7 nicotinic acetylcholine receptor allosteric modulators with close chemical similarity but diverse pharmacological properties, Mol. Pharm. 81 (2012) 710–718.

[43] J. Newcombe, A. Chatzidaki, T.D. Sheppard, M. Topf, N.S. Millar, Diversity of nicotinic acetylcholine receptor positive allosteric modulators revealed by mutagenesis and a revised structural model, Mol. Pharm. 93 (2018) 128–140.

[44] J.K. Gill, A. Chatzidaki, D. Ursu, E. Sher, N.S. Millar, Contrasting properties of α7-selective orthosteric and allosteric agonists examined on native nicotinic acetylcholine receptors, PLoS One 8 (1) (2013), e55047, https://doi.org/10.1371/journal.pone.0055047.

[45] N.A. Horenstein, R.L. Papke, A.R. Kulkarni, G.U. Chaturbhuj, C. Stokes, K. Manther, et al., Critical molecular determinants of α7 nicotinic acetylcholine receptor allosteric activation: separation of direct allosteric activation and positive allosteric modulation, J. Biol. Chem. 291 (2016) 5049–5067.

[46] C. Stokes, S. Garai, A.R. Kulkarni, L.N. Cantwell, C.M. Noviello, R.E. Hibbs, et al., Heteromeric neuronal nicotinic acetylcholine receptors with mutant β subunits acquire sensitivity to α 7-selective positive allosteric modulators, J. Pharm. Exp. Ther. 370 (2019) 252–268.

[47] J.J. Post-Munson, R.L. Pieschl, T.F. Molski, J.D. Graef, A.W. Hendricson, R.J. Knox, et al., B-973, a novel piperazine positive allosteric modulator of the α7 nicotinic acetylcholine receptor, Eur. J. Biochem 799 (2017) 16–25.

[48] S. Garai, K.S. Raja, R.L. Papke, J.R. Deschamps, M.I. Damaj, G.A. Thakur, B-973, a novel α7 nAChR ago-PAM: racemic and asymmetric synthesis, electrophysiological studies, and in vivo evaluation, ACS Med Chem. Lett. 9 (2018) 1144–1148.

[49] G.A. Thakur, A.R. Kulkarni, J.R. Deschamps, R.L. Papke, Expeditious synthesis, enantiomeric resolution, and enantiomer functional characterization of (4-(4-bromophenyl)-3a,4,5,9btetrahydro-3H-cyclopenta[c]quinoline-8-sulfonamide (4BP-TQS): An allosteric agonist-positive allosteric modulator of α7 nicotinic acetylcholine receptors, J. Med Chem. 56 (2013) 8943–8947.

[50] R.L. Papke, S. Garai, C. Stokes, N.A. Horenstein, A.D. Zimmerman, K.A. Abboud, et al., Differing activity profiles of the stereoisomers of 2,3,5,6TMP-TQS, a putative silent allosteric modulator of α 7 nAChR, Mol. Pharm. 98 (2020) 292–302.

[51] A. Gulsevin, R.L. Papke, C. Stokes, H.N.T. Tran, A.H. Jin, I. Vetter, et al., The allosteric activation of α7 nAChR by α-conotoxin MrIC Is modified by mutations at the vestibular site, Toxins 13 (2021) 555.

[52] M.S. Thomsen, J.D. Mikkelsen, Type I and II positive allosteric modulators differentially modulate agonist-induced up-regulation of α7 nicotinic acetylcholine receptors, J. Neurochem 123 (2012) 73–83.

[53] M. Hu, M. Gopalakrishnan, J. Li, Positive allosteric modulation of α7 neuronal nicotinic acetylcholine receptors: lack of cytotoxicity in PC12 cells and rat primary cortical neurons, Br. J. Pharm. 158 (2009) 1857–1864.

[54] D. Bertrand, C.-H. Lee, D. Flood, F. Marger, D. Donnelly-Roberts, Therapeutic potential of α7 nicotinic acetylcholine receptors, Pharm. Rev. 67 (2015) 1027–1073.

[55] H.J. Ng, E.R. Whittemore, M.B. Tran, D.J. Hogenkamp, R.S. Broide, T.B. Johnstone, et al., Nootropic α7 nicotinic receptor allosteric modulator derived from $GABA_A$ receptor modulators, Proc. Natl. Acad. Sci. USA 104 (2007) 8059–8064.

[56] D. Bertrand, S. Bertrand, S. Casser, E. Gubbins, J. Li, M. Gopalakrishnan, Positive allosteric modulation of the α7 nicotinic acetylcholine receptor: ligand interactions with distinct binding sites and evidence for a prominent role of the M2-M3 segment, Mol. Pharm. 74 (2008) 1407–1416.

[57] G.T. Young, R. Zwart, A.S. Walker, E. Sher, N.S. Millar, Potentiation of α7 nicotinic acetylcholine receptors via an allosteric transmembrane site, Proc. Natl. Acad. Sci. USA 105 (2008) 14686–14691.

[58] A. Taly, P.-J. Corringer, T. Grutter, L. Prado de Carvalho, M. Karplus, J.-P. Changeux, Implications of the quaternary twist allosteric model for the physiology and pathology of nicotinic acetylcholine receptors, Proc. Natl. Acad. Sci. USA 103 (2006) 16965–16970.

[59] N. Unwin, Refined structure of the nicotinic acetylcholine receptor at 4Å resolution, J. Mol. Biol. 346 (2005) 967–989.

[60] T. Collins, N.S. Millar, Nicotinic acetylcholine receptor transmembrane mutations convert ivermectin from a positive to a negative allosteric modulator, Mol. Pharm. 78 (2010) 198–204.

[61] T. Collins, G.T. Young, N.S. Millar, Competitive binding at a nicotinic receptor transmembrane site of two α7-selective positive allosteric modulators with differing effects on agonist-evoked desensitization, Neuropharmacol 61 (2011) 1306–1313.

[62] A. Chatzidaki, J.M. D'Oyley, J.K. Gill-Thind, T.D. Sheppard, N.S. Millar, The influence of allosteric modulators and transmembrane mutations on desensitisation and activation of α7 nicotinic acetylcholine receptors, Neuropharmacol 97 (2015) 75–85.

[63] C.L.C. Smelt, V.R. Sanders, J. Newcombe, R.P. Burt, T.D. Sheppard, M. Topf, et al., Identification by virtual screening and functional characterisation of novel positive and negative allosteric modulators of the α7 nicotinic acetylcholine receptor, Neuropharmacol 139 (2018) 194–204.
[64] D.F. Cully, P.S. Paress, K.K. Liu, J.M. Schaeffer, J.P. Arena, Identification of a *Drosophila melanogaster* glutamate-gated chloride channel sensitive to the antiparasitic agent avermectin, J. Biol. Chem. 271 (1996) 20187–20191.
[65] R.E. Hibbs, E. Gouaux, Principles of activation and permeation in an anion-selective Cys-loop receptor, Nature 474 (2011) 54–60.
[66] S.J. Lansdell, C. Sathyaprakash, A. Doward, N.S. Millar, Activation of human 5-hydroxytryptamine type 3 receptors via an allosteric transmembrane site, Mol. Pharm. 87 (2015) 87–95.
[67] M.M. Pałczyńska, M. Jindrichova, A.J. Gibb, N.S. Millar, Activation of α7 nicotinic receptors by orthosteric and allosteric agonists: influence on single-channel kinetics and conductance, Mol. Pharm. (2012) 910–917.
[68] M. Jindrichova, S.J. Lansdell, N.S. Millar, Changes in temperature have opposing effects on current amplitude in α7 and α4β2 nicotinic acetylcholine receptors, PLoS One (2012) 7, https://doi.org/10.1371/journal.pone.0032073.
[69] T.S. Tillman, E. Seyoum, D.M. Mowrey, Y. Xu, P. Tang, ELIC-α7 nicotinic acetylcholine receptor (α7nAChR) chimeras reveal a prominent role of the extracellular-transmembrane domain interface in allosteric modulation, J. Biol. Chem. 289 (2014) 13851–13857.
[70] R.L. Papke, N.A. Horenstein, A.R. Kulkarni, C. Stokes, L.W. Corrie, C.-Y. Maeng, et al., The activity of GAT107, an allosteric activator and positive modulator of α7 nAChR, is regulated by aromatic amino acids that span the subunit interface, J. Biol. Chem. 289 (2014) 4515–4531.
[71] R.L. Papke, C. Stokes, M.I. Damaj, G.A. Thakur, K. Manther, M. Treinin, et al., Persistent activation of α7 nicotinic ACh receptors associated with stable induction of different desensitized states, Br. J. Pharm. 175 (2018) 1838–1854.
[72] A. Gulsevin, R.L. Papke, C. Stokes, S. Garai, G.A. Thakur, M. Quadri, et al., Allosteric agonism of α 7 nicotinic acetylcholine receptors: receptor modulation outside the orthosteric site, Mol. Pharm. 95 (2019) 606–614.
[73] A.B. Smit, N.I. Syed, D. Schaap, J. van Minnen, J. Klumperman, K.S. Kits, et al., A glial-derived acetylcholine-binding protein that modulates synaptic transmission, Nature 411 (2001) 261–268.
[74] K. Brejc, W.J. van Dijk, R.V. Klaassen, M. Schuurmans, J. van der Oost, A.B. Smit, et al., Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors, Nature 411 (2001) 269–276.
[75] M. Quadri, S. Garai, G.A. Thakur, C. Stokes, A. Gulsevin, N.A. Horenstein, et al., Macroscopic and microscopic activation of α 7 nicotinic acetylcholine receptors by the structurally unrelated allosteric agonist-positive allosteric modulators (ago-PAMs) B-973B and GAT107, Mol. Pharm. 95 (2019) 43–61.
[76] R. Spurny, S. Debaveye, A. Farinha, K. Veys, A.M. Vos, T. Gossas, et al., Molecular blueprint of allosteric binding sites in a homologue of the agonist-binding domain of the α7 nicotinic acetylcholine receptor, Proc. Natl. Acad. Sci. USA 112 (2015) E2543–E2552.
[77] F. Delbart, M. Brams, F. Gruss, S. Noppen, S. Peigneur, S. Boland, et al., An allosteric binding site of the α7 nicotinic acetylcholine receptor revealed in a humanized acetylcholine-binding protein, J. Biol. Chem. 293 (2018) 2534–2545.
[78] G. Hassaine, C. Deluz, L. Grasso, R. Wyss, M.B. Tol, R. Hovius, et al., X-ray structure of the mouse serotonin 5-HT_3 receptor, Nature 512 (2014) 276–281.
[79] M. Brams, C. Govaerts, K. Kambara, K.L. Price, R. Spurny, A. Gharpure, et al., Modulation of the Erwinia ligand-gated ion channel (ELIC) and the 5-HT3 receptor via a common vestibule site, eLife 9 (2020), e51511.
[80] H.R. Arias, M.P. Blanton, α-Conotoxins, Int J. Biochem Cell Biol. 32 (2000) 1017–1028.
[81] A.-H. Jin, I. Vetter, S. Dutertre, N. Abraham, N.B. Emidio, M. Inserra, et al., MrIC, a novel α-conotoxin agonist in the presence of PNU at endogenous α7 nicotinic acetylcholine receptors, Biochem 53 (2014) 1–3.
[82] A. Mueller, H. Starobova, M.C. Inserra, A.-H. Jin, J.R. Deuis, S. Dutertre, et al., α-Conotoxin MrIC is a biased agonist at α7 nicotinic acetylcholine receptors, Biochem Pharm. 94 (2015) 155–163.
[83] M. Stefan, N. Le Novère, Cooperative binding, PLoS Comput. Biol. 9 (2013), e1003106.
[84] C. Bohr, K. Hasselbalch, A. Krogh, Ueber einen in biologischer beziehung wichtigen einfluss, den die kohlensäurespannung des blutes auf dessen sauerstoffbindung übt, Skand. Skand. Arch. Physiol. 16 (1904) 402–412.
[85] J. Monod, J. Wyman, J.-P. Changeux, On the nature of allosteric transitions: a plausible model, J. Mol. Biol. 12 (1965) 88–118.