Allosteric Agonism of α7 Nicotinic Acetylcholine Receptors: Receptor Modulation Outside the Orthosteric Site

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ABSTRACT
Nicotinic acetylcholine receptors (nAChRs) are members of the Cys-loop superfamily of ligand-gated ion channels. Typically, channel activation follows the binding of agonists to the orthosteric binding sites of the receptor. α7 nAChRs have a very low probability of channel activation, which can be reversed by the binding of α7 selective positive allosteric modulators (PAMs) to putative sites within the transmembrane domains. Although typical PAMs, like PNU-120596, require coapplication of an orthosteric agonist to produce large channel activations, some, like GAT107 and B-973B ([S]-3-[3,4-difluorophenyl]-N-(1-[6-(4-(pyridin-2-yl)piperazin-1-yl)pyrazin-2-yl]ethyl)propanamide), are characterized as allosterically activating PAMs, which also bind to an allosteric activation (AA) site in the extracellular domain and activate the α7 ion channel by themselves. We previously characterized N,N-diethyl-N’-phenylpiperazine analogs with various functions. In this work, we docked members of this family to a homology model of the α7 receptor extracellular domain. The compound 1,1-diethyl-4-(naphthalene-2-yl)piperazin-1-ium (2NDEP) a weak partial agonist, showed particularly favorable docking and binding energies at the putative AA site of the receptor. We hypothesized that 2NDEP could couple with PAMs through the AA site. This hypothesis was tested with the α7 mutant C190A, which is not activated by orthosteric agonists but is effectively activated by GAT107. The results showed that 2NDEP acts as an allosteric agonist of α7C190A when coapplied with the PAM PNU-120596. Also, the allosteric activity was nearly abolished upon coapplication with the AA site-selective antagonist 2,3,5,6MP-TQS (cis-trans-4-(2,3,5,6-tetramethylphenyl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline-8-sulfonamide), consistent with AA site involvement. Overall, our findings show a novel mode of agonism through an allosteric site in the extracellular domain of α7 nAChR.

Introduction
The high expression of the α7 type nicotinic acetylcholine receptor (nAChR) in brain regions such as the hippocampus commends it as a potential target for cognitive disorders such as Alzheimer’s disease, and schizophrenia. More recently, its essential role in the cholinergic anti-inflammatory pathway (CAP) has suggested that it may also provide a path for the treatment of inflammatory diseases and neuropathic pain. However, the unique properties of α7 nAChR continue to present puzzles, challenges, and opportunities.

The first well-studied nAChR was that of the neuromuscular junction. A pentamer of α1, β1, γ, and either δ or ε subunits, with two α1 subunits per pentamer, muscle-type nAChRs function efficiently as ligand-gated ion channels mediating fast synaptic transmission (for review, see Papke, 2014). The nAChR of autonomic ganglia, containing varying assemblies of α3, β2, β4, α5, and possibly other subunits, also are effective mediators of synaptic transmission. Although heteromeric assemblies of nAChR subunits expressed in the brain, such as α4, β2, α5, α6, and β3, are capable of synaptic-like activation in response to the rapid application of high concentrations of ACh, the slower, more diffuse delivery of ACh in the brain is consistent with neuromodulatory functions of these receptors in vivo. For all of these heteromeric nAChR subtypes, ion channel function relies largely on the binding of ACh or other agonists to the two orthosteric ligand binding domains configured at the interface between α and non-α subunits.

α7 nAChRs, though, function most effectively as homomeric pentamers with five α subunits, and hence potentially five agonist binding sites at each α-α interface. However, the
binding of agonist to the orthosteric activation (OA) sites of α7 nAChR couples rather ineffectively to channel activation, and high levels of agonist binding primarily induce nonconducting (i.e., desensitized) conformations. Despite these limitations, as noted above, α7 nAChRs have been identified as essential activators of CAP, a sort of metabotropic cascade mediated by immune cells, for which α7 channel activation seems unimportant. This is consistent with the data that show that silent agonists, which are partial agonists with little or no efficacy for channel activation in neuronal cells or in heterologous expression systems, are among the best activators of CAP (Horenstein and Papke, 2017).

The discovery of α7 selective positive allosteric modulators (PAMs) has led to important insights into the conformational dynamics of α7 nAChRs. The most effective PAMs bind within the transmembrane domain (TMD) of the receptor and destabilize one or more of the nonconducting states induced by the binding of agonists or silent agonists, resulting in protracted bursts of channel activation in just a small fraction of the receptors when an orthosteric agonist is also bound. Although conventional PAMs like PNU-120596 and 2,3,5,6MP-TQS require the agonist induction of the PAM-sensitive desensitized states, allosteric activating PAMs (ago-PAMs), like GAT107 (Fig. 1) and B-973 [(S)-3-(3,4-difluorophenyl)-N-(1-6-(4-pyridin-2-yl)piperazin-1-yl)pyrazin-2-yl]ethyl propanamide] (Gill et al., 2011; Thakur et al., 2013; Post-Munson et al., 2017), have also been identified. Ago-PAMs induce channel activation without orthosteric agonist–induced conformational effects and can indeed activate large currents in receptors with mutations in the orthosteric binding interface that disable orthosteric agonist binding and channel activation [i.e., nonorthostERICaly activatable receptors (NOARs)]. Several lines of evidence have indicated that these ago-PAMs produce direct allosteric activation (AA) by acting at both the conventional transmembrane PAM site and an additional allosteric site in the extracellular domain (ECD) of the receptor, including inhibition by molecules such as 2,3,5,6MP-TQS (Fig. 1) that do not affect OA (Gill-Thind et al., 2015; Horenstein et al., 2016). This suggested the hypothesis that alternative small ligands might be identified that would bind to such an allosteric agonist binding site to produce PAM-dependent activation.

As noted above, we have previously identified silent agonists as PAM-dependent activators that hypothetically worked through the orthosteric agonist binding site, including a large structurally diverse group of N,N-diethyl-N'-phenylpiperazine (dEPP)–related compounds (Quadri et al., 2016). We conducted in silico analyses to test the hypothesis that one or more of these analogs might alternatively bind preferentially to the AA site to produce PAM-dependent activation. We confirmed that 1,1-diethyl-(4-naphthalene-2-yl)piperazin-1-ium (2NDEP) (Fig. 1), a compound that showed particularly favorable docking and binding energies at the putative AA site of the receptor, produced PAM-dependent activation that did not depend on binding to a functional orthosteric binding site. These results confirm the presence of a novel AA site on α7 that can be selectively targeted. Developing new drugs for this site may also provide a new approach for the control of CAP and associated indications related to neuropathic pain and inflammation.

Fig. 1. Structures of the three molecules used for the docking and MD and electrophysiology studies: the putative allosteric agonist 2NDEP, AA site-selective antagonist 2,3,5,6MP-TQS, and ago-PAM GAT107.

Materials and Methods

Computational

A full description of computational methodology is provided in the Supplemental Material. Briefly, all compounds that were used in docking studies had their structures optimized and atomic parameters calculated with the Gaussian09 and antechamber programs (Wang et al., 2004, 2006; Frisch et al., 2016). The α7 nAChR homology model (Quadri et al., 2019) used for docking was created with Prime (Schrödinger, LLC, Cambridge, MA) (Jacobson et al., 2002), using as a template the crystal structure for the humanized acetylcholine (ACh) binding protein (Protein Data Bank identification number 3SQ6) (Li et al., 2011). The homology model was refined using molecular mechanics and molecular dynamics (MD) within the AMBER 16 suite of programs (AMBER, University of California, San Francisco, San Francisco, CA) (Case et al., 2008).

Docking studies used the GlideXP (Schrödinger, LLC) (Friesner et al., 2006) program within the Schrödinger computational suite. Binding energies were calculated with the molecular mechanics Poisson–Boltzmann surface area (MMPBSA) method, within AMBER 16.

Experimental

Chemicals and Reagents. ACh and buffer chemicals were purchased from Sigma-Aldrich (St. Louis, MO). The diEPP derivative 2NDEP was synthesized in our laboratory as described previously (Quadri et al., 2016). GAT107 and 2,3,5,6MP-TQS were synthesized by G.A.T. (Northeastern University, Boston, MA) after the published procedures (Kulkarni and Thakur, 2013; Thakur et al., 2013; Gill-Thind et al., 2015). PNU-120596 was synthesized in our laboratory (University of Florida, Gainesville, FL) by Dr. Kinga Chojnacka after the published procedure (Hurst et al., 2005).

Heterologous Expression of nAChRs in Xenopus laevis Oocytes. The human α7 nAChR clone was obtained from Dr. J. Lindstrom (University of Pennsylvania, Philadelphia, PA). The human RIC-3 (resistance-to-cholinesterase 3) clone was obtained from Dr. M. Treinin (Hebrew University, Jerusalem, Israel) and coinjected with α7 to improve the level and speed of α7 receptor expression without affecting the pharmacological properties of the receptors (Halevi et al., 2003). Subsequent to linearization and purification of the plasmid cDNAs, complementary RNAs were prepared using the Ambion mMESSAGE mMACHINE in vitro RNA Transcription Kit (Thermo Fisher Scientific, Waltham, MA). The α7C190A mutant was made as previously described with a C116S double mutation to prevent spurious disulfide bond formation with the free cysteine (Papke et al., 2011). The harvested oocytes were treated with 1.4 mg/ml type 1 collagenase (Worthington Biochemicals, Freehold, NJ) for 2–4 hours at room temperature in calcium-free Barth’s solution (88 mM NaCl, 1 mM KCl, 2.38 mM NaHCO3, 0.82 mM MgSO4, 15 mM HEPES, and 12 mg/l tetracycline, pH 7.6) to remove the ovarian tissue and the
follicular layers. Stage V oocytes were subsequently isolated and injected with 50 nl of 5–20 ng nAChR subunit complementary RNA. Oocytes were maintained in Barth’s solution with calcium [additional 0.32 mM Ca(NO3)2 and 0.41 mM CaCl2], and recordings were carried out 1–14 days after injection.

**Two-Electrode Voltage-Clamp Electrophysiology.** Experiments were conducted using OpusXpress 6000A (Molecular Devices, Union City, CA) (Papke and Stokes, 2010). Both the voltage and current electrodes were filled with 3 M KCl. Oocytes were voltage clamped at −60 mV at room temperature (24°C). The oocytes were bath perfused with Ringer solution (115 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl2, 10 mM HEPES, and 1 mM ATP, pH 7.2) at 2 ml/min. To evaluate the effects of experimental compounds compared with ACh-evoked responses of various nAChR subtypes expressed in oocytes, control responses were defined as the average of two initial applications of ACh made before test applications. Solutions were applied from 96-well plates via disposable tips. Drug applications were 12 seconds in duration followed by 181-second washout periods. A typical recording for each set of oocytes constituted two initial control applications of ACh, one or more experimental compound applications, and then a follow-up control application of ACh. The control ACh concentration was 60 μM for the wild-type (WT) receptor experiments, and the average of independent 10 μM GAT107 responses was used as the control for the NOAR experiments. The responses were calculated as both peak current amplitudes and net responses.

**Results**

We used docking, MD simulations, and MMPBSA binding energy calculations for in silico screening of diEPP compounds that behaved as silent or partial agonists, as reported in the study by Quadri et al. (2016), to identify potential candidates for allosteric activators. Because there is no high-resolution structure of α7 available, we built a homology model of the ECD of the α7 nAChR based on the high-resolution crystal structure of the humanized chimeric ACh binding protein (α7 AChBP) (Li et al., 2011), which has 64% sequence identity to the α7 ECD. The α7 nAChR homology model was constructed without imposing fivefold symmetry, and the MD simulations that were run to refine the homology model enhanced the asymmetry. Considering the asymmetry of the OA sites and the past reports of binding sites in the vestibule of the receptor and its analog α7 AChBP (Spurny et al., 2015; Horenstein et al., 2016; Delbart et al., 2018; Quadri et al., 2019), we docked the ligands into all five interfaces of the receptor and its analog α7 AChBP with a grid large enough to cover the vestibular side to ensure that all possible binding sites and multiple subunit configurations were included in our analysis.

Docking of 16 diEPP derivatives (Supplemental Fig. 2) to the WT α7 ECD model showed ligand poses at the traditional OA site under the C-loop of the receptor and at another region formed by the vestibular loops of the neighboring receptor subunits (Fig. 2, top row). The base of this vestibular site was formed by the residues 89–93 and 98–106, and the top part was formed by the residues 101–106 of the neighboring interface. Other residues contributing to the bottom of the cavity were F33, L56, Q57, M58, I90, and L91. This region observed for the diEPP derivatives is consistent with the putative AA site, as defined in our previous
of the 16 compounds analyzed, only exclusively preferred docking into the AA sites at ICD and IDE OA site poses (Supplemental Fig. 2, bottom row).

Based on these data, we chose 2NDEP as the likeliest candidate to act through the AA site, with lower OA site preference, and tested its activity experimentally compared with the ago-PAM GAT107.

In these experiments, our working model and assumptions were that: 1) 2NDEP was able to bind to the putative AA site, and 2) it would require a PAM to yield detectable channel activation. Thus, we wanted to use a compound known to inhibit AA, but it would be necessary to show that the blockade of any 2NDEP activity was occurring at the AA site, not the PAM site. For this purpose, we used 2,3,5,6MP-TQS (Fig. 1) to specifically block AA site activity of 2NDEP.

The selectivity of 2,3,5,6MP-TQS for the AA site has been established in past studies (Gill et al., 2012; Horenstein et al., 2016). Note that the washout of GAT107 does not cause a total loss of PAM activity due to tight binding at the TMD PAM site of the α7 receptor (Papke et al., 2014). This phenomenon is called “primed potentiation.” The inhibitory effect of 2,3,5,6MP-TQS on AA but not primed potentiation has been used to substantiate the selectivity of its inhibitory effects at the AA site rather than the PAM site (Horenstein et al., 2016). In the experiments reported here, 300 μM 2,3,5,6MP-TQS had a clear inhibitory effect on AA, but primed potentiation was not significantly affected, as could be expected from an AA site–selective antagonist. Therefore, we confirmed the previous observation that selective AA site inhibition by 2,3,5,6MP-TQS was valid for our experimental conditions as well (Fig. 3A).

As a key experiment, we used 2,3,5,6MP-TQS to block the activation by 2NDEP and ACh potentiated by the type II PAM PNU-120596 (Hurst et al., 2005; Grønlien et al., 2007) to see whether 2NDEP was affected more by the application of 2,3,5,6MP-TQS than were the ACh responses, as would be expected from an allosteric agonist. The 60 μM ACh gave moderate activation when applied by itself, which significantly increased by 30 μM PNU-120596, peak currents increasing 11.1 ± 3.6-fold (P < 0.001) and a net charge 46.2 ± 13.5-fold (P < 0.001). Coapplication of 300 μM 2,3,5,6MP-TQS with 60 μM ACh plus 30 μM PNU-120596 resulted in a partial (60%) inhibition of the PNU-120596–potentiated ACh response (Fig. 3B, left panel; Table 1). Next, we tested the effect of 2,3,5,6MP-TQS on 2NDEP activity. As expected from previous results (Quadri et al., 2016), the application of 30 μM 2NDEP gave a weak partial agonist response when applied by itself, with peak currents and a net charge of ±10% that of ACh applied alone. Coapplication of 30 μM 2NDEP with 30 μM PNU-120596 resulted in large potentiated responses (peak currents and net charge responses were 5.4 ± 0.9-fold and 10.8 ± 3.2-fold larger than ACh control subjects, respectively), which were almost completely abolished by 300 μM 2,3,5,6MP-TQS (Fig. 3B, right panel; Table 1), in support of significant AA site involvement in 2NDEP activity. Note that the waveforms of these responses are the result of several complex underlying processes. In the examples shown in Fig. 3B, there are two or three ligands binding to multiple sites on each receptor. The receptors are distributed all over the surface of the oocyte, and drugs are being delivered in such a way that the concentration of each ligand increases and then decreases. In contrast to the ACh/PNU responses, the later peaks in the presence of 2NDEP are actually occurring as the drug concentrations are falling. 2NDEP is probably a channel blocker (as reported for other drugs in that series) (Quadri et al., 2016), and so the second peak is likely to represent relief of some of that channel block.
To further focus on the involvement of the AA site as a possible epitope for 2NDEP activity, we used a NOAR (Horenstein et al., 2016). NOARs are α7 nAChRs with mutations in the OA site, such that the receptors do not respond to ACh or other orthosteric agonists, but they can be allosterically activated by ago-PAMs such as GAT107. This provides a tool to discern whether a component of 2NDEP activity occurs independent of the OA site (Papke et al., 2014; Horenstein et al., 2016). Because the NOAR α7C190A lacks the critical vicinal disulfide in the C-loop of the OA site, it cannot be activated by orthosteric ligands even when coupled with high concentrations of PNU-120596 (Fig. 4A). This allowed us to use α7C190A to test whether 2NDEP could act as an allosteric agonist through the AA site of this mutant.

2NDEP did not activate the NOAR α7C190A when applied by itself (our limit of detection is 10 nA), but coapplication of 100 μM 2NDEP with 100 μM PNU-120596 to α7C190A evoked substantial responses (peak currents, 12.9 ± 1.9 μA), which were significantly (P < 0.001) inhibited by the coapplication of 300 μM 2,3,5,6MP-TQS (Table 1), consistent with allosteric agonism of this molecule through the AA site (Fig. 4B). Note that for this mutant, the two peaks are still apparent in the potentiated responses, especially in the 2,3,5,6MP-TQS–inhibited response, when both 2NDEP and the allosteric agonist are declining in concentration.

Fig. 3. (A) Inhibition of the 10 μM GAT107 response on WT α7 nAChR by coapplication with 300 μM 2,3,5,6MP-TQS had little effect on the primed potentiation response. (Top trace) Responses to 60 μM ACh, 10 μM GAT107, and 60 μM ACh on the same set of cells, with 12-second drug applications 3.5 minutes apart (n = 7). (Bottom trace) Responses to 60 μM ACh, 10 μM GAT107 plus 300 μM 2,3,5,6MP-TQS, and 60 μM ACh on the same set of cells, with 12-second drug applications 3.5 minutes apart (n = 7). The black line is the average and gold bands are the S.E. (B, left panel) Activation of the WT α7 nAChR by 60 μM ACh plus 30 μM PNU-120596 (n = 8) (black lines with yellow error bands) and the effect of 300 μM 2,3,5,6MP-TQS on potentiated response (n = 5) (blue lines with cyan error bands). (Right panel) Activation of the WT α7 nAChR by 30 μM 2NDEP plus 30 μM PNU-120596 (n = 8) (black lines with yellow error bands) and the effect of 300 μM 2,3,5,6MP-TQS on potentiated response (n = 8) (blue lines with cyan error bands). These averaged traces can be directly used to compare peak currents, as illustrated in Supplemental Fig. 1, and additionally convey the kinetic features of the responses and intrinsic variability over time. For details and statistics, see Table 1.
Based on the effectiveness of 2NDEP in activating α7C190A when coapplied with PNU-120596, we tested other available diEPP derivatives with this mutant to find other potential allosterically active compounds (Fig. 5A). The results showed that p-CF₃ diEPP, another compound that docked to AA sites at both the ICD and IDE interfaces of the WT receptor, could activate the α7C190A mutant in a 2,3,5,6MP-TQS–sensitive manner at a level comparable to 2NDEP (Fig. 5B, left panel). However, p-CF₃ diEPP behaved differently than 2NDEP in the WT α7 experiments. Contrary to 2NDEP, 2,3,5,6MP-TQS–enhanced p-CF₃ diEPP potentiated activity in WT α7 experiments rather than diminishing it (Fig. 5B, right panel). These results suggest that p-CF₃ diEPP prefers to bind to the OA site of the WT receptor, but in the absence of a functional OA site it can also couple with PAMs through the AA site, as evident in α7C190A results.

To summarize, 2NDEP and p-CF₃ diEPP, hypothesized to be allosterically active through our docking and binding energy calculations, were experimentally confirmed to be allosteric activators of the α7 receptor. 2NDEP or p-CF₃ diEPP plus PNU-120596 showed potentiated responses at α7C190A, and 2,3,5,6MP-TQS, which targets the AA site, inhibited...
PNU-120596–potentiated 2NDEP and p-CF₃ diEPP responses. However, the behavior of the two molecules were different at the WT receptor such that blocking the AA site with 2,3,5,6MP-TQS abolished 2NDEP activity while enhancing p-CF₃ diEPP activity.

**Discussion**

The diEPP family of α₇ ligands were originally designed based on the nonselective orthosteric ganglionic nAChR agonist dimethylphenylpiperazinium, and they have been shown to act as α₇ antagonists, partial agonists, and silent agonists depending on the substitution of the phenyl ring or the nature of the charged group (Quadri et al., 2016, 2017; Horenstein and Papke, 2017). In the present work, we identified the allosteric agonism of a diEPP derivative, 2NDEP, formerly reported as a weak partial agonist of α₇ nAChR. PNU-120596–potentiated responses of 2NDEP by the WT α₇ were nearly abolished by the AA site-selective antagonist 2,3,5,6MP-TQS. 2NDEP could not activate the NOAR α₇C190A by itself, but it showed a robust potentiated response when coapplied with PNU-120596, which was also sensitive to 2,3,5,6MP-TQS.

Our homology model of the pentameric α₇ ECD was not strictly fivefold symmetric, due to the differences in conformation between individual subunits, and the asymmetry was retained throughout the MD simulations. In addition to the asymmetry within the pentamer, an important finding was that the five potential OA sites of the α₇ receptor are not equivalent. This nonequivalence was evident in the distribution of binding sites at the ECD and the differences in binding configuration of the ligands at the OA and putative AA sites of each interface (Supplemental Table 1). Asymmetry of the α₇ receptor has been reported in past MD studies with the α₇ receptor models that contain only ECD and both ECD and TMD domains (Henchman et al., 2003; Law et al., 2005; Chiodo et al., 2017; Quadri et al., 2019). Crystal structures of different α₇ ligands with AChBP also reflect the variations in ligand binding configurations and OA site geometries. Different AChBP crystal structures cocrystallized with strychnine, GTS-21, or 4OH-GTS-21 show at least two distinct ligand binding configurations (Hibbs et al., 2009; Brams et al., 2011). Nonequivalent sites may be the result of stochastic fluctuations or they may be functionally significant. Heteromeric receptors have been shown to have nonorthodox binding sites at α/α and β/α subunit interfaces that play regulatory roles (Wang and Lindstrom, 2018). By a similar logic, nonequivalence of the α₇ subunit interfaces may play a role in the modulation of desensitization and the formation of allosteric or regulatory binding sites at the ECD of the receptor (Gulsevin, 2017). On the other hand, the identification of the exact role of this nonequivalence will require longer simulation times and extensive sampling of the protein conformations. But in the present study, we explored the various binding modes enabled by nonequivalent binding sites and interfaces.

The docking of GAT107 and 2,3,5,6MP-TQS to the WT α₇ ECD model showed both the OA and AA site poses despite the lack of experimental evidence connecting the two molecules to the OA site (data not shown). These poses at the OA site may...
reflect weak ligand binding that would eventually lead to dissociation, or they may represent stable binding configurations with no functional importance since these molecules cannot form π-cation or electrostatic interactions with important aromatic cage residues such as W149, due to their lack of a permanent positive charge. Contrarily, the charged nitrogen of 2NDEP did form π-cation interactions with the aromatic cage, but the naphthalene functional group adopted multiple orientations at the interfaces, whereas the smaller acetyl group of ACh pointed toward the hydrophobic pocket inside the OA site formed by the residues L109 and L119.

The inhibitory effects of 2,3,5,6MP-TQS were predominantly at the AA site, but we also observed the inhibition of the PNU-120596–potentiated ACh responses of WT a7 (Fig. 3B, left panel). There are a couple of possibilities to explain this curiosity. One explanation is that 2,3,5,6MP-TQS binds to the PAM binding site at the TMD of the receptor to nonspecifically block the potentiating effects of PNU-120596. This is possible, considering the structural similarity between 2,3,5,6MP-TQS and TQS-derivative PAMs, but the weak effect of 2,3,5,6MP-TQS on primed potentiation by GAT107 after a washout period (Fig. 3A) suggests that a transition of the receptor to the type II PAM-sensitive desensitization state can be achieved without a functional OA site, therefore opening the door to the development of drugs that target allosteric sites on the ECD of the a7 receptor with little or no activity at the OA site. Alternatively, a7 ligands that can bind to either OA or AA sites may show better therapeutic effect when coapplied with an AA site–selective antagonist.

In summary, we used docking, MD, and binding energy calculations to predict ligands that target an allosteric binding site in the vestibule of the a7 ECD. These predictions were supported experimentally with voltage-clamp electrophysiology, and we thus identified 2NDEP as an allosteric agonist of the a7 nAChR that can activate the a7 NOAR C190A when coapplied with PNU-120596 in a 2,3,5,6MP-TQS–sensitive manner. Additionally, 2NDEP may serve as a lead for the future development of new ligands that could have activity in the CAP and therefore potential for therapeutic applications.

**Authorship Contributions**

**Participated in research design:** Gulsevin, Papke, Quadri, Horenstein.

**Conducted experiments:** Gulsevin, Stokes, Quadri.

**Contributed new reagents or analytic tools:** Gulsevin, Garai, Thakur, Quadri.

**Performed data analysis:** Gulsevin, Papke, Stokes.

**Wrote or contributed to the writing of the manuscript:** Gulsevin, Papke, Stoeck, Quadri, Horenstein.

**References**


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