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Distinct Profiles of α7 nAChR Positive Allosteric Modulation Revealed by Structurally Diverse Chemotypes

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ABSTRACT

Selective modulation of α7 nicotinic acetylcholine receptors (nAChRs) is thought to regulate processes impaired in schizophrenia, Alzheimer's disease, and other dementias. One approach to target α7 nAChRs is by positive allosteric modulation. Structurally diverse compounds, including PNU-120596, 4-naphthalene-1-yl-3*a*,4,5,9*b*-tetrahydro-3-*H*-cyclopenta[*c*]quinoline-8-sulfonic acid amide (TQS), and 5-hydroxyindole (5-HI) have been identified as positive allosteric modulators (PAMs), but their receptor interactions and pharmacological profiles remain to be fully elucidated. In this study, we investigated interactions of these compounds at human α7 nAChRs, expressed in *Xenopus laevis* oocytes, along with genistein, a tyrosine kinase inhibitor. Genistein was found to function as a PAM. Two types of PAM profiles were observed. 5-HI and genistein predominantly affected the apparent peak current (type I) whereas PNU-120596 and TQS increased the apparent peak current and evoked a distinct weakly decaying current (type II). Concentration-responses to agonists [ACh, 3-[(3*E*)-3-[(2,4-dimethoxyphenyl)methylidene]-5,6-dihydro-4*H*-pyridin-2-yl]pyridine dihydrochloride (GTS-21), and *N*-[(3*R*)-1-azabicyclo[2.2.2]oct-3-yl]-4-chlorobenzamide hydrochloride (PNU-282987)] were potentiated by both types, although type II PAMs had greater effects. When applied after α7 nAChRs were desensitized, type II, but not type I, PAMs could reactivate α7 currents. Both types of PAMs also increased the ACh-evoked α7 window currents, with type II PAMs generally showing larger potentiation. None of the PAMs tested increased nicotine-evoked Ca^{2+} transients in human embryonic kidney 293 cells expressing human α4β2 or α3β4 nAChRs, although some inhibition was noted for 5-HI, genistein, and TQS. In summary, our studies reveal two distinct α7 PAM profiles, which could offer unique opportunities for modulating α7 nAChRs in vivo and in the development of novel therapeutics for central nervous system indications.

Nicotinic acetylcholine receptors (nAChRs) belong to the pentameric superfamily of ligand-gated ion channels that includes $5HT_3$, $GABA_A$, and glycine receptors. Twelve neuronal nicotinic subunits have been identified thus far (α2–α10; β2–β4) of which nine subunits, α2–α7 and β2–β4, predominate in the mammalian brain (Paterson and Nordberg, 2000). Multiple functionally distinct nAChR complexes can be assembled either as homomeric functional pentamers, such as α7 nAChRs (Couturier et al., 1990), or as heteropentamers with at least two different subunits, such as α4β2 nAChRs (Gotti et al., 2006).

The role of α7 nAChRs in the CNS has received much attention since their discovery (Couturier et al., 1990). These subunits, when expressed in heterologous expression systems, activate and desensitize rapidly and, furthermore, exhibit relatively higher calcium permeability compared with other nAChR combinations (Dajas-Bailador and Wonnacott, 2004). In the brain, the α7 subunit is distributed at high levels, including in regions involved in learning and memory, hippocampus and cerebral cortex (Rubboli et al., 1994; Wevers et al., 1994; Breese et al., 1997). At the cellular level, activation of α7 nAChRs is thought to regulate interneuron

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ABBREVIATIONS: nAChR, nicotinic acetylcholine receptor; CNS, central nervous system; PNU-282987, *N*-[(3*R*)-1-azabicyclo[2.2.2]oct-3-yl]-4-chlorobenzamide hydrochloride; PAM, positive allosteric modulator; PNU-120596, 1-(5-chloro-2,4-dimethoxy-phenyl)-3-(5-methyl-isoxazol-3-yl)-urea; 5-HI, 5-hydroxyindole; TQS, 4-naphthalen-1-yl-3*a*,4,5,9*b*-tetrahydro-3H-cyclopenta[*c*]quinoline-8-sulfonic acid amide; compound 6, *N*-(4-chlorophenyl)-α-[[(4-chloro-phenyl)amino]methylene]-3-methyl-5-isoxazoleacet-amide; MLA, methyllycaconitine; PP2, protein phosphatase 2; SU6656, 2-oxo-3-(4,5,6,7-tetrahydro-1*H*-indol-2-ylmethylene)-2,3-dihydro-1*H*-indole-5-sulfonic acid dimethylamide; FLIPR, fluorometric imaging plate reader; NMDG, *N*-methyl-D-glucamine; BAPTA-AM, 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid, acetoxymethyl ester; AR-R17779, (5*S*)-spiro[1,3-oxazolidine-5,8′-1-azabicyclo[2.2.2]octane]-2-one.

excitability (Frazier et al., 1998), modulate the release of excitatory and inhibitory neurotransmitters (Alkondon et al., 2000), and contribute to neuroprotective effects in experimental in vitro models of cellular damage (Levin and Rezvani, 2002). Antisense (Curzon et al., 2006) and more recent gene knock-out studies (Wehner et al., 2004; Keller et al., 2005) have demonstrated that α7 nAChRs could play important roles in certain cognitive and attentive tasks. For example, α7 nAChR genetic knockout mice have shown impaired performance in ethanol-induced contextual fear conditioning (Wehner et al., 2004) and showed further deterioration in hippocampus-selective associative learning and memory when crossed with Tg2576 animals (Dineley et al., 2005). Selective α7 nAChR agonists such as PNU-282987 (Hajós et al., 2005), PHA-543613 (Wishka et al., 2006), and AR-R17779 (Felix and Levin, 1997; Van Kampen et al., 2004) improve performance in sensory gating, novel object recognition, social recognition, water maze performance, or inhibitory avoidance models of cognitive function. Given these roles, targeting α7 nAChRs has been considered as a viable strategy for a variety of diseases involving cognitive deficits and neurodegeneration (for review, see Levin and Rezvani, 2002; Gotti et al., 2006).

An alternate approach to enhance α7 nAChR function is by augmenting effects of the neurotransmitter ACh via positive allosteric modulation that could reinforce the endogenous cholinergic neurotransmission without directly activating α7 nAChRs. Indeed, such positive allosteric modulator (PAM) approach to enhance channel activity has been proven clinically successful for $GABA_A$ receptors (Hevers and Luddens, 1998). The preclinical validation of α7 nAChR PAMs will require selective compounds yet to be identified because many of the compounds identified so far are weak, nonselective or incompletely characterized pharmacologically. Various molecules have been reported to positively modulate α7 nAChR, including PNU-120596 (Hurst et al., 2005), 5-hydroxyindole (5-HI) (Zwart et al., 2002), ivermectin (Krause et al., 1998), galantamine (Samochocki et al., 2003), bovine serum albumin (Conroy et al., 2003), SLURP-1 (Chimienti et al., 2003), an acetylcholinesterase derived peptide (Zbarsky et al., 2004), (2-amino-5-keto)thiazole compounds (Broad et al., 2006), and compound 6 (Ng et al., 2007). Among these compounds, PNU-120596 and compound 6 improved auditory gating and other cognitive deficits (Hurst et al., 2005; Ng et al., 2007), supporting the concept that α7 nAChR PAMs may be effective in vivo. Genistein, a nonselective kinase inhibitor (Akiyama et al., 1987) has been shown to increase α7 responses (Charpantier et al., 2005; Cho et al., 2005). Although there was evidence that effects of genistein could be mediated through kinase inhibition, direct allosteric modulatory effects on α7 nAChR may be involved, and detailed studies aimed at identifying direct effects of genistein on α7 nAChR have yet to be carried out.

This study describes the pharmacological profiles of structurally diverse PAMs: 5-HI, PNU-120596, and TQS. In addition, evidence is presented that genistein also functions as an α7 nAChR PAM. The effects of these compounds were determined on recombinant α7 current evoked by diverse α7 agonists [ACh, GTS-21 (de Fiebre et al., 1995), and PNU-282987 (Bodnar et al., 2005)] as well as on $\alpha4\beta2$ and $\alpha3\beta4$ nAChRs to investigate their selectivity. Our results demonstrate the existence of at least two types of α7 nAChR PAMs based upon differential effects on current responses, reactivation of desensitized α7 nAChRs, augmentation of ACh window current, and agonist concentration-response characteristics. This study provides an insight into the understanding of PAM actions relevant to the design of novel compounds with potential therapeutic utility in diseases such as: Alzheimer's disease, schizophrenia, and attention deficit hyperactivity disorder, where α7 nAChRs are thought to play important roles.

Materials and Methods

Materials. Oocytes were obtained from adult female *Xenopus laevis* frogs (Blades Biological Ltd., Cowden, Edenbridge, Kent, UK) and cared for in accordance with the Institutional Animal Care and Use Committee guidelines. Genistein, 5-hydroxindole, herbimycin A, ACh, nicotine, choline, MLA, and BAPTA-AM were obtained from Sigma (St. Louis, MO). GTS-21 and staurosporine were purchased from Tocris (London, UK). PP2 and SU6656 were obtained from Biaffin GmbH and Co KG (Kassel, Germany). PNU-120596, TQS, and PNU-282987 were synthesized in-house. All other chemicals and reagents were obtained from Sigma or Fisher Scientific (Essex, UK).

Two-Electrode Voltage-Clamp on *X. laevis* Oocytes. *X. laevis* oocytes were prepared for electrophysiological experiments as described previously (Briggs et al., 1995; Briggs and McKenna, 1998). In brief, three to four lobes from ovaries of female adult *X. laevis* frogs were removed and defolliculated after treatment with collagenase type 1A (2 mg/ml; Sigma) prepared in low-Ca^{2+} Barth's solution [90 mM NaCl, 1.0 mM KCl, 0.66 mM $NaNO_3$, 2.4 mM $NaHCO_3$, 10 mM HEPES, 2.5 mM sodium pyruvate, 0.82 mM $MgCl_2$, and 0.5% (v/v) penicillin-streptomycin solution, pH = 7.55 (Sigma)] for 1.5 to 2 h at ~18°C under constant agitation to obtain isolated oocytes. The oocytes were injected with ~4 to 6 ng of human α7 nAChR cRNA, kept at 18°C in a humidified incubator in modified Barth's solution [90 mM NaCl, 1.0 mM KCl, 0.66 mM $NaNO_3$, 2.4 mM $NaHCO_3$, 10 mM HEPES, 2.5 mM sodium pyruvate, 0.74 mM $CaCl_2$, 0.82 mM $MgCl_2$, 0.5% (v/v) penicillin-streptomycin solution, pH 7.55] and used 2 to 7 days after injection. Responses were measured by two-electrode voltage clamp using parallel oocyte electrophysiology test station (Abbott, Abbott Park, IL) (Trumbull et al., 2003). During recordings, the oocytes were bathed in Ba^{2+}-OR2 solution (90 mM NaCl, 2.5 mM KCl, 2.5 mM $BaCl_2$, 1.0 mM $MgCl_2$, 5.0 mM HEPES, and 0.0005 mM atropine, pH 7.4) to prevent activation of Ca^{2+}-dependent currents and held at −60 mV at room temperature (~20°C). Modulators were given for ~60 s before agonist application. Agonists were applied for 1 s at 6 ml/s with or without modulators to the recording chambers. The buffer flow to the chamber, however, did not resume until at least 3 s had passed. The parallel oocyte electrophysiology test station system, similar to any other electrophysiological setup using *X. laevis* oocytes, cannot apply α7 agonists fast enough to cause rapid and complete activation of α7 channels without desensitization; hence, the measured maximum peak current responses underestimate the maximum achievable current mediated by α7 nAChRs. For this reason, we use the term *apparent peak current* to describe the maximum observed peak current amplitude response. In inhibition experiments, which were carried out as part of the window current analysis, a three-step protocol was used. In the first addition, 1 mM ACh without PAM was applied to obtain a control response. In the second addition, different concentrations of ACh were given in the presence of 3 μM PNU-120596, 5 μM TQS, 50 μM genistein, 1 mM 5-HI, or no PAM (buffer control) for 10 min. After this preincubation, 1 mM ACh in the continual presence or absence of PAM was applied for at least 3 s. This protocol allowed for normalization of the concentration-inhibition curves to 1 mM ACh without PAM. The agonist responses obtained in the presence or absence of PAM were also normalized to 1 mM ACh without PAM ensuring that the same control condition was used in comparing the

window current effects. In current-voltage experiments aimed at identifying reversal potentials for initial and secondary components, α7 currents were evoked by 100 μM ACh in the presence of 1 μM TQS while changing the holding potential from −140 to +80 mV in steps of 20 mV and normalized to the respective initial and secondary current responses measured at −100 mV taken as −1.0 for each cell.

Calcium Imaging. Functional activities were assessed in human embryonic kidney 293 cell lines expressing human α4β2 or α3β4 subunits by measuring intracellular calcium changes using a fluorometric imaging plate reader (FLIPR; Molecular Devices, Sunnyvale, CA). Cells were plated at densities of 25 to 60 × 10^3 cells/well in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum in 96-well clear-bottomed, black-walled plates precoated with poly(D-lysine) (75 μl/well of 0.01 g/l solution ≥30 min) and allowed to incubate for 24 to 48 h at 37°C in 5% CO_2 in a humidified environment. After aspirating the media, cells were incubated for ~45 to 60 min with Fluo-4 AM calcium indicator dye in the dark at room temperature (Invitrogen, Carlsbad, CA) dissolved in *N*-methyl-D-glucamine (NMDG)/Ringer buffer (140 mM NMDG, 5 mM KCl, 1 mM $MgCl_2$, 10 mM HEPES, and 10 mM $CaCl_2$, pH 7.4). After dye loading, cells were gently washed with the same buffer removing extracellular dye, leaving ~100 μl/well after the final wash. Cells were placed in the FLIPR chamber, where 50 μl of 3× stock concentration of test modulators or buffer prepared in the same NMDG/Ringer buffer were added to the wells in the first addition for 5 min. In the second addition, also for 5 min, 50 μl of 4× stock concentrations of nicotine (3–10 μM) or buffer were added.

Data Analysis. In two-electrode voltage-clamp studies, responses were quantified by measuring apparent peak current amplitude. Apparent peak current responses were expressed as percentage response to 100 μM ACh when assessing PAM responses or to 1 mM ACh when determining agonist concentration responses. In inhibition experiments, the concentration-responses to preapplied ACh concentrations in the presence or absence of PAM were plotted against the observed effects on 1 mM ACh without PAM as explained above. In calcium imaging studies, raw fluorescence data were corrected by subtracting fluorescence values from wells with buffer only added. Peak fluorescent responses were determined using FLIPR software and expressed as -fold increases over the submaximum nicotine response (3–10 μM, corresponding to EC_{30} to EC_{50}); 1-fold indicates no change in the response. Data were analyzed and fitted using Prism (GraphPad Software, San Diego, CA). Sigmoidal dose-response (variable slope) function was used to fit the replicates. The pEC_{50} ($-\log EC_{50}$) or pIC_{50} ($-\log IC_{50}$) values and associated S.E.M. values were obtained from fitted results. The maximum mean ± S.E.M. values were calculated from individual experiments. $p < 0.05$ was considered statistically significant. Student's *t* test (Microsoft Excel; Microsoft Corp., Redmond, WA) was used to compare data sets.

Results

Modulation of α7 nAChRs by PNU-120596, TQS, and 5-HI. First, the effects of representative compounds from structurally diverse chemotypes, including 5-HI (Zwart et al., 2002), PNU-120596 (Hurst et al., 2005), and TQS (Becker et al., 2006) were assessed on α7 function as agonists and then as PAMs (see Fig. 1 for structures). None of these compounds alone induced activation of α7 currents up to the maximum concentrations tested (30 μM except for 5-HI, which was tested up to 10 mM) indicating that they are not α7 agonists. Under similar conditions, ACh and α7 selective agonists such as PNU-282987 were effective in evoking currents (see *Modulation of Agonist Concentration-Responses by PAMs*). When 5-HI, PNU-120596, and TQS were added to the cells during preincubation and then α7 currents were obtained by submaximal concentration of ACh (100 μM), concentration-dependent potentiation of current responses was obtained. As shown in Figs. 2 and 3, the rank order of potency, based on apparent peak current amplitude analysis, was PNU-120596 (pEC_{50} = 5.8 ± 0.09) > TQS (pEC_{50} = 5.5 ± 0.07) > 5-HI (pEC_{50} = 3.2 ± 0.06). A similar rank order of potency was obtained when total current charge (integral or area under the current response) was analyzed.

PAMs had qualitatively different effects on ACh responses, as exemplified by the traces depicted in Fig. 2, and could be classified into two types. PNU-120596 and TQS dramatically increased the apparent peak current response and seemed to reduce the current decay rate (designated as type II). At highest concentrations tested, these compounds in the presence of ACh evoked a nondecaying or weakly decaying current during the recording interval (usually 3 s). Typically, at lower concentrations of PNU-120596 (e.g., 1 μM; Fig. 2a, trace B) and TQS (e.g., 1 μM; Fig. 2b, trace B), the effects on the amplitude were minimal and an apparent secondary component with amplitude similar to that of the initial apparent peak was identifiable. The onset of this secondary component was clearly distinct from that of the initial component. With increasing concentrations of these PAMs, the apparent peak and secondary components overlapped, producing an apparent single current profile with relatively rapid onset and very weak current decay. During washout, when both agonist and PAM were removed, it typically took 50 to 100 s for TQS (≥10 μM) and longer than 200 to 250 sec for PNU-120596 (≥10 μM) for the holding current to return to pretreatment levels, suggesting relatively prolonged effects.

In contrast, 5-HI (and genistein see below) predominantly increased α7 nAChR apparent peak amplitude response without robustly affecting current decay rate (designated as type I). Although the decay rate could have been slightly altered, especially at the highest concentrations tested (Fig. 2c, traces C and D); the effects, however, were modest. Furthermore, unlike PNU-120595 and TQS, there was no secondary component identifiable with onset separate from that of the initial apparent peak component. This suggests that the mechanism by which 5-HI allosterically potentiates α7 nAChR response is distinct from that mediated by PNU-120596 and TQS.

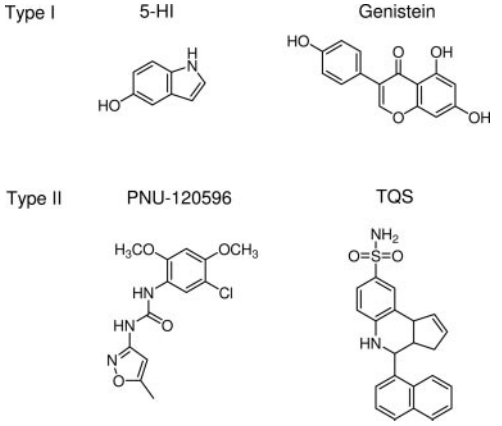

Fig. 1. Diversity of α7 nAChR PAMs. Depicted are structures of PNU-120596, TQS, genistein, and 5-HI.

To further characterize the nature of the ACh-evoked secondary component, current-voltage experiments were carried out in which the holding potential was varied from −140 up to +80 mV, and ACh evoked α7 currents measured in the presence of 1 μM TQS (see Fig. 4). At this concentration of TQS, both initial and secondary components are easily separable. As shown, both initial and secondary current components reversed at ~0 mV consistent with their being mediated directly by α7 nAChR.

Mechanism of α7 nAChR Modulation by Genistein. Genistein is a nonspecific kinase inhibitor (Akiyama et al., 1987) that also increases α7 nAChR current response. This effect has been attributed to inhibition of Src kinase, although a direct mechanism involving positive allosteric modulation may be involved (Charpantier et al., 2005; Cho et al., 2005). To examine whether effects of genistein are due to positive allosteric modulation, three types of experiments were conducted: 1) pre- and coapplication of genistein and ACh to test onset of effects; 2) comparison with other kinase inhibitors; and 3) interaction with 5-HI, another type I PAM.

When added directly, genistein did not activate α7 nAChR current up to the maximum tested concentration of 300 μM (n = 10). In the continued preincubation with genistein, the apparent peak current α7 response was potentiated in a concentration-dependent manner with a pEC_{50} value of 4.6 ± 0.1 and maximum potentiation of ~ 2.6-fold (Figs. 2d and 3). When preincubation was eliminated and genistein was simply coapplied with ACh, to limit time for potential kinase-mediated effects to develop, genistein was still effective in potentiating the α7 nAChR response. The degree of potentiation was ~2.2-fold without preincubation (Fig. 5a) compared with ~2.6-fold with preincubation (Fig. 3), hence ~15% less. We also examined the effect of 5-HI under preapplication and coapplication conditions with ACh and determined that this compound exhibited ~35% lesser potentiation when coapplied. The -fold increases were ~ 5.4 (Fig. 3) and 3.5 (Fig. 5a) for pre- and coapplication conditions, respectively.

Staurosporine and herbimycin A, two nonspecific kinase inhibitors (Yanagihara et al., 1991; Zakar et al., 1999), were also tested to determine their effects on α7 currents. Oocytes were exposed to these two inhibitors for 5- to 10- or 60-min preincubation followed by ACh application. At both time points, staurosporine (up to 30 nM) and herbimycin A (up to 10 μM) failed to increase or inhibit the α7 currents evoked by ACh (n ≥2) (see Fig. 5c). When genistein was coapplied together with ACh after short- or long-term exposure to either staurosporine (30 nM) or herbimycin A (10 μM), the maximum potentiation of the current was similar to that of genistein alone (see Fig. 5b for example). This observation supports a direct allosteric effect of genistein because this compound was still able to increase α7 currents, even though intracellular kinases were inhibited by the treatment with staurosporine or herbimycin A. In addition, we studied the effects of PP2 and SU6656, two Src tyrosine kinase inhibitors that increased α7 currents in one study (Charpantier et al., 2005) but not in another (Cho et al., 2005). In this study, PP2 and SU6656, similarly to herbimycin A and staurosporine, had no effect on α7 nAChRs (see Fig. 5c). These experiments also support a direct allosteric effect of genistein. We rationalized that if genistein effects on α7 currents were due primarily to inhibition of protein kinases, then staurospor-

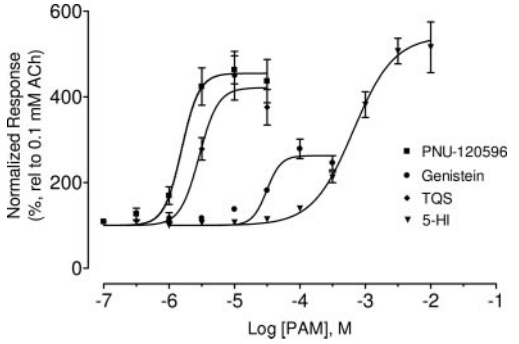

Fig. 3. Summary of PAM concentration-responses potentiating submaximum ACh evoked α7 currents by PNU-120596, TQS, 5-HI, and genistein. The respective mean pEC_{50} and maximum efficacy values are 3.2 ± 0.1 and 541 ± 26% for 5-HI, 4.7 ± 0.11 and 267 ± 16% for genistein, 5.5 ± 0.2 and 418 ± 25% for TQS, and 5.8 ± 0.1 and 455 ± 20% for PNU-120596. The n value for each data point is n = 5 to 12.

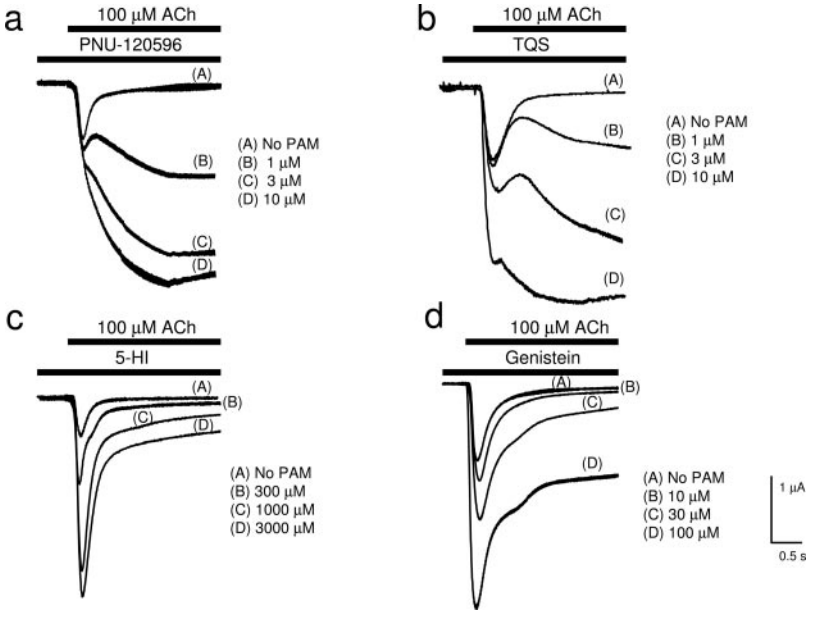

Fig. 2. Enhancement of ACh-evoked α7 responses by PNU-120596, TQS, 5-HI, and genistein. Representative traces showing the effects of PNU-120596 (a), TQS (b), 5-HI (c), and genistein (d). Concentrations of the modulators are indicated on the right within each panel. The horizontal bars indicate when 100 μM ACh was added in the presence or absence of the specified concentration of PAM. The holding potential was −60 mV.

ine, herbimycin A, PP2, or SU6656 should mimic the effects of genistein, and they should abolish or attenuate the increased current responses to genistein. As shown, the data support the contrary hypothesis that genistein effects are primarily mediated by a direct allosteric effect on the α7 nAChR.

Finally, the interaction of genistein and 5-HI, both type I PAMs, was evaluated by exposing oocytes to a nearly fully efficacious concentration of either 5-HI or genistein followed by determination of the concentration-dependent effects of the other modulator. As shown in Fig. 5d, the net modulatory effect of 5-HI was attenuated by pretreatment with 50 μM genistein. Likewise, the effect of genistein was occluded by pre-exposure with 3 mM 5-HI. This lack of additivity is consistent with the hypothesis that the 5-HI and genistein act through a similar mechanism, but the results do not exclude the possibility that the nonadditivity was due to a ceiling effect such that either compound alone could exert the maximum possible effect.

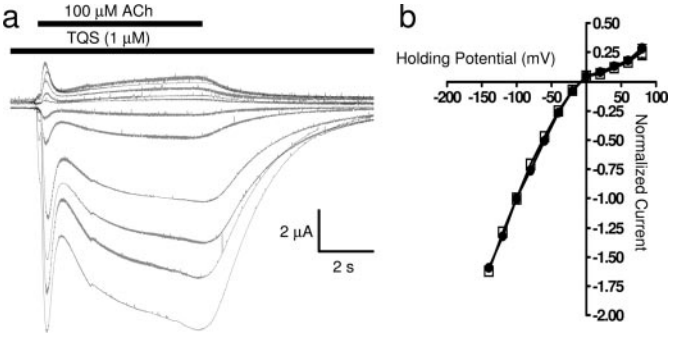

Fig. 4. Current-voltage relationship for ACh evoked initial and secondary α7 component responses in the presence of TQS. a, representative current traces obtained by varying the holding potentials from −140 to +60 mV in steps of 20 mV. For each voltage, ACh (100 μM) was applied in the presence of TQS (1 μM). The interval between the traces was at least 3 min. b, the mean current-voltage relationship for the ACh evoked initial and secondary components (n = 2). The responses were normalized to −100 mV for each cell (taken as the normalized current of −1.0 at this voltage) and illustrate that both initial and secondary components reverse at ~0 mV consistent with both being mediated directly by α7 nAChR.

Differential Reactivation of Desensitized α7 nAChR by PAMs. To investigate the effects of modulators on desensitized α7 nAChRs, oocytes were first exposed to 100 μM ACh for at least 60 s (and up to 5 min) to desensitize the channels. Subsequently, in the continued presence of ACh, modulators were applied (for a 4-min interval) followed by washout of the modulator and ACh. As exemplified by Fig. 6, the addition of either 100 μM genistein (n = 4) or 3 mM 5-HI (n = 4) caused no change in current responses. However, when 3 μM PNU-120596 (n = 4) or 5 μM TQS (n = 4) was added, there was an increase in the α7 current. This indicates that modulators that affect both apparent peak current response and evoke the secondary component are able to re-activate currents when α7 channels are desensitized. On the other hand, 5-HI and genistein, neither of which evokes the secondary component, do not exhibit this property.

Experiments were also done in presence of MLA, an α7 antagonist, or BAPTA-AM, a membrane permeable intracellular Ca^{2+} chelator. MLA at 100 nM completely abolished the ability of 3 μM PNU-120596 or 5 μM TQS ($n \geq 2$) to reactivate desensitized α7 nAChR in the presence of ACh (data not shown). In BAPTA-AM experiments, oocytes were incubated with 100 μM BAPTA-AM for at least 3 h, allowing for sufficient chelation of cytosolic Ca^{2+}. Both 3 μM PNU-120596 and 5 μM TQS ($n \geq 2$) were able to reactivate α7 channels without obvious differences in responses, whether treated with BAPTA-AM or not (data not shown). These experiments indicate that the reactivated current by type II PAMs in oocytes is indeed mediated by α7 nAChRs and unaffected by chelation of intracellular Ca^{2+} and related Ca^{2+} dependent currents such as those mediated Ca^{2+} dependent Cl^{-} channels.

Modulation of Agonist Concentration-Responses by PAMs. Next, we evaluated the effects of α7 modulators on current responses to different α7 agonists. Modulators were preincubated at a fixed concentration (corresponding to ~

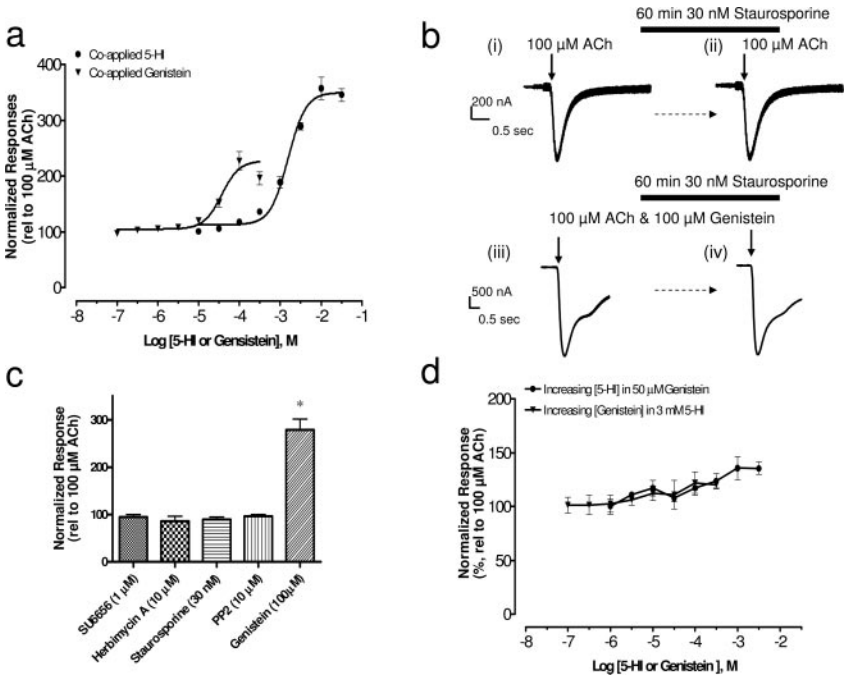

Fig. 5. Potentiation of α7 nAChR currents by genistein involves direct effects. a, the concentration-responses to 5-HI and genistein added as coapplication without any preincubation. The respective mean pEC_{50} and maximum efficacy values are 2.8 ± 0.1 and 350 ± 7% for 5-HI and 4.4 ± 0.1 and 227 ± 10% for genistein; each data point is n = 4 to 6. b, representative α7 current traces obtained before and after prolonged treatment with staurosporine for either ACh alone or ACh with genistein treatment. Currents in response to 100 μM ACh in traces i and ii are before and after, respectively, 60-min treatment with 30 nM staurosporine. Currents in response to 100 μM ACh and 100 μM genistein in traces iii and iv are before and after, respectively, 60-min treatment with 30 nM staurosporine. c, the effects of different tyrosine kinase inhibitors on α7 nAChR currents after at least 5-min preincubation. Among these inhibitors, only genistein potentiated the current evoked by 100 μM ACh. Each data point is $n \geq 4$, * indicates $p < 0.05$. d, the concentration responses to potentiate α7 currents by 5-HI in the presence of nearly maximal concentration of genistein and by genistein in the presence of nearly maximal concentration of 5-HI. Each data point is n = 3.

EC_{70-80} in modulator concentration-response experiments: 3 μM PNU-120596, 5 μM TQS, 50 μM genistein, and 1 mM 5-HI) followed by determination of agonist concentration-responses in the continued presence of the modulator. In particular, we aimed to compare the effects of the different modulators on concentration responses to full agonists, ACh and PNU-282987 (Bodnar et al., 2005), and a partial α7 agonist, GTS-21 (de Fiebre et al., 1995). As summarized in Table 1 and Figs. 7 and 8, the modulators affected the concentration-responses to all three agonists by shifting the potencies and increasing the maximum responses. The highest enhancement in efficacy was observed with GTS-21. In the absence of any PAM, GTS-21 behaved as a weak partial agonist ($pEC_{50} < 3$, max = 26.5% at 300 μM). However, in the presence of any of the four modulators, GTS-21 became more efficacious, with maximum responses in the range of ~70 to 170%. Table 1 also indicates that 5-HI and genistein affected the agonist potencies to a lesser extent than did PNU-120596 and TQS, differences ranging from 0.2 to 0.6-log units. For example, for ACh (pEC_{50} of 3.9 without any modulator), 5-HI and genistein shifted the potency by 0.3 to 0.4 log units, whereas PNU-120596 and TQS shifted by 0.8 to 0.9 log units. This suggests that modulators affecting both apparent peak current and secondary component generation are more likely to shift the concentration-response profile to α7 agonists to a greater extent than would modulators altering only the apparent peak current response.

Effect of PAMs on ACh Window Current. It is well established that α7 nAChRs are activated and desensitized by agonists. In fact, the constants for half-maximum inhibition or desensitization (pIC_{50}) are 1 or 2 orders of magnitude higher that those for activation (pEC_{50}), resulting in a very minimal window current (i.e., the overlap between the activation and inactivation) (Briggs and McKenna, 1998). The effects of modulators on the ACh window current were therefore examined. The activation curves to ACh, discussed above and summarized in Fig. 9 and Table 1, showed differential abilities of PAMs to shift the potencies and efficacies. In contrast, the PAMs did not seem to have any significant effect on the ACh concentration-inhibition curves (see Fig. 9). As shown, the pIC_{50} for the inactivation curve of ACh was 5.1 ± 0.05 (n = 4). In the presence of TQS, PNU-120596, genistein, and 5-HI, the pIC_{50} values were 4.9 ± 0.03 (n = 3), 4.8 ± 0.04 (n = 4), 4.7 ± 0.1 (n = 3), and 4.8 ± 0.04 (n = 3), respectively. As a measure of the window currents, we have calculated the integral of the overlapping area of the inhibition and activation curves for the different modulators tested. For ACh, the normalized ratios in the absence of any modulator and in the presence of genistein, 5-HI, TQS, and PNU-120596 were 1 (control, ACh alone), 6, 7, 12, and 17, respectively. This analysis indicates that PNU-120596 and TQS, both type II PAMs, produced more robust effects on ACh window currents than 5-HI and genistein, both type I PAMs.

Effects of PAMs on $\alpha4\beta2$ and $\alpha3\beta4$ nAChRs. To investigate the selectivity of modulation, the effects of 5-HI, genistein, TQS, and PNU-120596 were measured in recombinant human embryonic kidney 293 nAChR cell lines expressing either human $\alpha4\beta2$ or human $\alpha3\beta4$ using submaximum concentrations of nicotine (3–10 μM) to evoke Ca^{2+} transients. Genistein, TQS, or PNU-120596 alone did not affect basal Ca^{2+} in either of the two cell lines. 5-HI up to 1 mM had no effect. However, at 3 and 10 mM, it alone transiently decreased fluorescence in both cell lines followed by a slow recovery in the signal. Overall, submaximum nicotine evoked Ca^{2+} signals were not increased by the tested PAMs. PNU-120596 produced only small ~0.1- to 0.2-fold decreases in the nicotine-evoked Ca^{2+} signals in the two cell lines tested (Table 2). TQS also produced a small maximum reduction of ~0.1-fold on $\alpha3\beta4$ responses and a decrease of ~0.7-fold in $\alpha4\beta2$ responses. Likewise, genistein and 5-HI decreased nicotine-evoked signals mediated by the two subunit combinations by ~0.4- to 0.8-fold. Hence, modulators at the concentrations showing positive effects on α7 function did not potentiate or increase Ca^{2+} signals mediated by $\alpha3\beta4$ and $\alpha4\beta2$ nAChRs. However, at comparable or higher concentra-

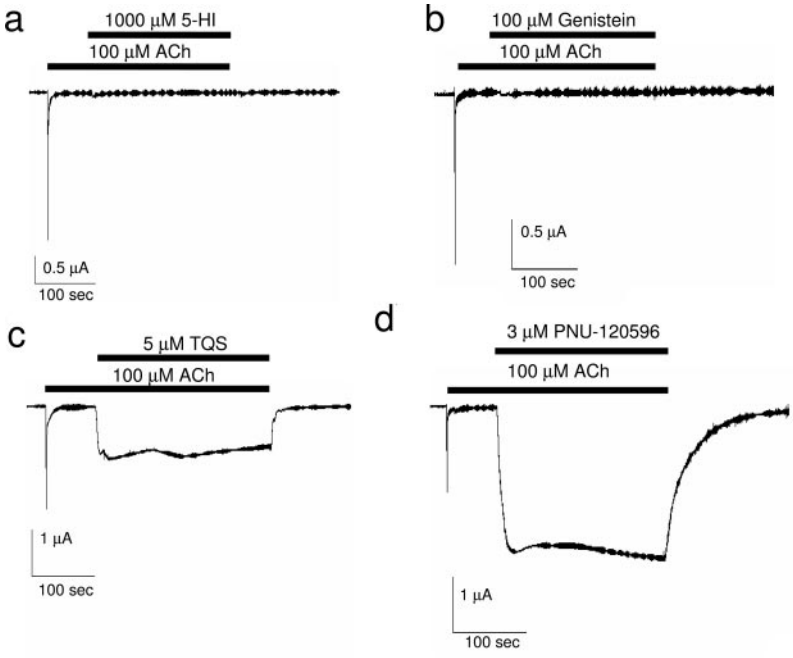

Fig. 6. Preferential activation of desensitized α7 nAChRs by type II PAMs. a and b, the effects of 5-HI and genistein added after ACh treatment indicating their inability to affect desensitized channels. c and d, the effects of TQS and PNU-120596, respectively. Compounds were added during the intervals indicated by the horizontal bars.

tions inhibition of nicotine evoked Ca^{2+} signals at both $\alpha4\beta2$ or $\alpha3\beta4$ subunits were observed except for PNU-120596.

Discussion

This study compares and contrasts the properties of four structurally distinct α7 nAChR modulators—PNU-120596, TQS, 5-HI, and genistein—and demonstrates important distinctions in their pharmacological profiles. We extend earlier observations made with PNU-120596 and 5-HI (Zwart et al., 2002; Hurst et al., 2005) and provide evidence that genistein effects on α7 nAChR are due primarily to a positive allosteric mechanism rather than via inhibition of protein kinases. We also characterize for the first time the properties of TQS as an α7 PAM. All four compounds increased currents evoked by α7 nAChR agonists. Based on their profiles, two types were recognized. Type I PAMs, exemplified by genistein and 5-HI, predominantly affected the apparent peak current response. Type II PAMs, illustrated by PNU-120596 and TQS, increased the apparent peak current amplitude and strongly evoked the secondary component with onset distinguishable from the initial apparent peak component especially at lower concentrations. Both types exhibit differential properties. Type II modulators were able to reactivate desensitized α7 nAChRs, whereas type I did not. The former had also greater effects on the α7 activation concentration-response curves. The ACh inhibition curves for α7 currents were affected similarly by type I and II compounds resulting in greater ACh window current effects by PNU-120596 and TQS rather than genistein and 5-HI. None of the four compounds, at concentrations active on α7, potentiated $\alpha4\beta2$ and $\alpha3\beta4$ nAChRs indicating that positive allosteric effects are selective for the α7 subtype.

Genistein Is a PAM of α7 nAChR. Genistein, a tyrosine kinase inhibitor, has been shown to increase α7 currents expressed in *X. laevis* oocytes, in rat hippocampus brain slice interneurons, and stably expressed in SH-SY5Y neuroblastoma cells (Charpantier et al., 2005; Cho et al., 2005). We have confirmed this increase of α7 currents. However, the mechanism underlying this effect remains controversial. In one study, evidence was provided for genistein causing rapid up-regulation of α7 receptors at the cell surface membrane (Cho et al., 2005). In contrast, another report identified no changes in cell surface labeling on neurons with ^{125}I-α-bungarotoxin (Charpantier et al., 2005). Genistein coapplication with agonist was found to be either effective (Charpantier et al., 2005) or ineffective (Cho et al., 2005) in potentiating α7 currents. Effects of genistein have been interpreted to occur via tyrosine dephosphorylation of non-α7 nAChR protein(s) rather than direct allosteric effect on α7 nAChR. Effects of PP2, another tyrosine kinase inhibitor, on α7 currents are

TABLE 1
Potencies and efficacies of ACh, GTS-21, and PNU-282987 on α7 currents in the absence or presence of test concentrations of PAMs

	Test Concentration	Acetylcholine			GTS-21			PNU-282987		
		pEC_{50}	Max	Hill Slope	pEC_{50}	Max	Hill Slope	pEC_{50}	Max	Hill Slope
	μM		%			%			%	
		3.9 ± 0.05	101 ± 4.3	1.6	<4.0	—[a]		5.6 ± 0.12	77 ± 5.6	1.1
5-HI	1000	4.2 ± 0.04	206 ± 5.5	1.6	4.8 ± 0.04	81 ± 2.4	1.9	6.3 ± 0.05	155 ± 4.8	1.5
Genistein	50	4.3 ± 0.05	167 ± 5.1	1.6	4.6 ± 0.11	67 ± 6.9	1.4	6.3 ± 0.04	105 ± 2.7	1.7
PNU-120596	3	4.8 ± 0.04	331 ± 8.9	2.8	5.1 ± 0.03	167 ± 7.1	2.9	6.7 ± 0.07	188 ± 8.3	2.1
TQS	5	4.7 ± 0.04	151 ± 3.3	1.8	5.0 ± 0.04	106 ± 8.4	2.9	6.7 ± 0.25	155 ± 22.1	1.7

[a] At the highest tested concentration, 300 μM, the response was 26.5 ± 3.9%. The *n* values are 3 to 9.

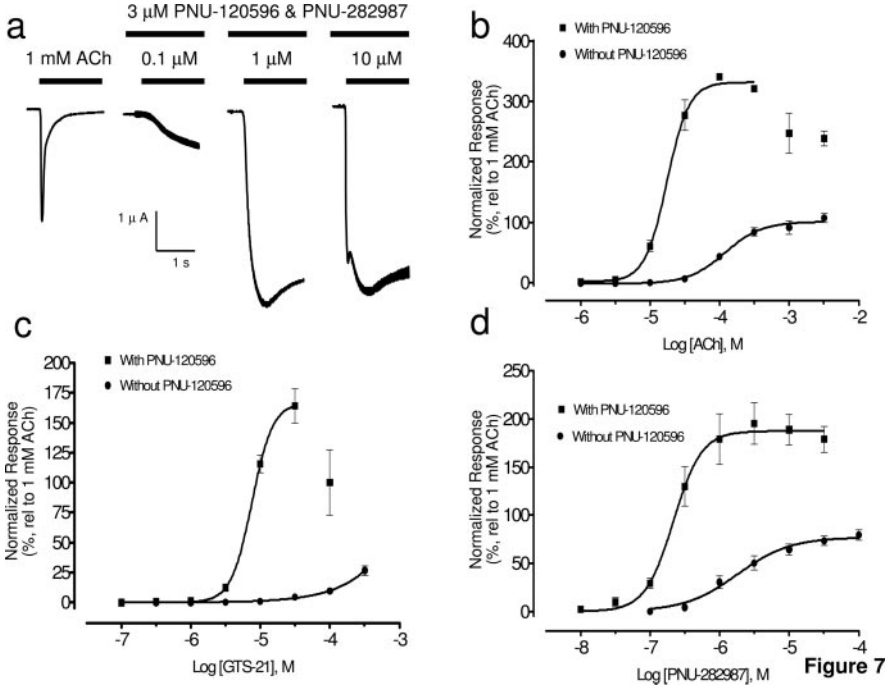

Fig. 7. Enhancement of α7 agonist responses by PNU-120596. a, representative traces in *X. laevis* oocytes expressing α7 evoked by ACh (1 mM, normalizing control) or in the presence of PNU-120596 (3 μM) for PNU-282987 (0.1, 1, and 10 μM) added as indicated by the horizontal bars. b, c, and d, the concentration responses to ACh, GTS-21, and PNU-282987, respectively, in the presence or absence of PNU-120596 (3 μM). Each data point is $n = 3$ to 6. Summary of potency and maximum efficacy is given in Table 1.

also inconclusive, with no (Cho et al., 2005) or potentiating (Charpantier et al., 2005) effects reported. In our study, genistein produced effects similar to those of 5-HI under similar testing conditions consistent with a direct allosteric modulation of α7 nAChR on the basis of several lines of evidence. First, genistein was effective when coapplied with ACh, indicating that pre-exposure to genistein was not required. Second, only genistein among the kinase inhibitors studied (staurosporine, herbimycin A, PP2, or SU6656), examined at concentrations showing effective kinase inhibition (Yanagihara et al., 1991; Hanke et al., 1996; Zakar et al., 1999; Blake et al., 2000), increased α7 currents. Third, the pretreatment with other tyrosine kinases inhibitors did not abolish or attenuate the modulatory effect of genistein. Fourth, the modulatory effect of genistein on α7 nAChR was occluded by effective concentrations of 5-HI and vice versa. This indicates that either both compounds bind to the same modulatory binding site or that there are two separate modulatory sites and activation of either is sufficient to allosterically potentiate α7 nAChR to a certain level that cannot be surpassed by activation of the other modulatory site.

Distinct Profiles of nAChR PAMs: Type I and II. The electrophysiological analysis of the effects of PAMs indicates that there are at least two distinct modulator profiles: type I, exemplified by 5-HI and genistein, and type II, exemplified by PNU-120506 and TQS. The primary difference between these two types is in their ability to evoke the secondary component. At high concentrations of type II PAMs, the initial and secondary components overlap, producing an apparent single complex. In the study by Hurst et al. (2005), the effects of PNU-120596 were judged to occur by slowing down the current decay rate. In this study, the concentration-responses to PNU-120596 and TQS show that at lower concentrations, there are two separate identifiable components. An initial component similar in time course to that of α7 agonists evoked in the absence of any PAM, and a secondary nondecaying or weakly decaying current component, which activates with a slower onset. With increasing concentrations of PAMs, the time courses overlap.

The concept of distinct PAM profiles has been postulated earlier although no previous study has compared and demonstrated such differences. For example, 5-HI (Zwart et al., 2002), ivermectin (Krause et al., 1998), galantamine (Samochocki et al., 2003), bovine serum albumin (Conroy et al., 2003), SLURP-1 (Chimienti et al., 2003), (2-amino-5-keto)thiazole compounds (LY2087101, LY1078733, and LY2087133) (Broad et al., 2006), and compound 6 (Ng et al., 2007) have been reported as α7 PAMs exhibiting profile characteristic of type I PAMs. PNU-120596 has been shown to exhibit a different profile (Hurst et al., 2005). This compound increased the apparent peak α7 current response and robustly affected the time course of current response. At the single-channel level, PNU-120596 increased mean open time, had no effect on ion selectivity, and had relatively little effect on unitary conductance (Hurst et al., 2005). PNU-120596 also increased ACh-evoked GABAergic synaptic activity recorded in pyramidal cells (Hurst et al., 2005) similar to effects of 5-HI in interneurons in hippocampus slices (Mok and Kew, 2006). 5-HI also enhanced ACh-stimulated glutamate evoked postsynaptic currents in cerebellar slices (Zwart et al., 2002) illustrating α7 PAM effects on synaptic activity. In this study, we showed that PNU-120596 potentiated the α7 currents expressed in *X. laevis* oocytes with an EC_{50} value of 1.6 μM (or pEC_{50} of 5.8) and maximum poteniation of ~4.5-fold, and we demonstrate that TQS is also a type II PAM exhibiting potency and efficacy similar to PNU-120596.

Comparison of Pharmacological Properties of Type I and II PAMs. Our study provides further insight into the pharmacological properties of type I and II PAMs. Compounds belonging to both types were effective in shifting the potencies of agonists to the left and in increasing their maximum efficacies, although type II PAMs were generally more effective (see Table 1). These changes are comparable with those reported by others for 5-HI (Zwart et al., 2002) and

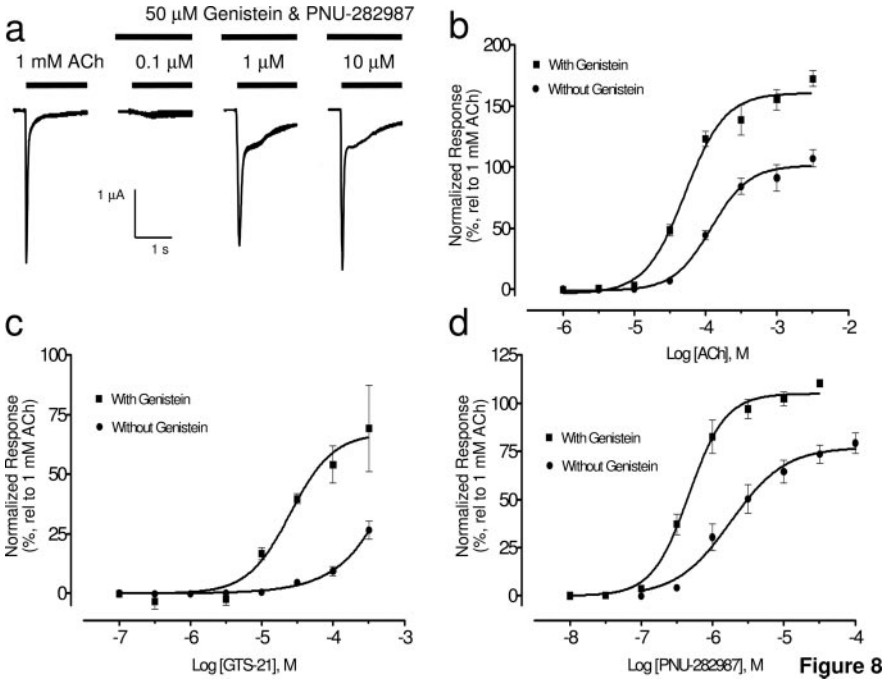

Figure 8

Fig. 8. Enhancement of α7 responses by genistein. (a) shows representative traces in *X. laevis* oocytes expressing α7 evoked by ACh (1 mM, normalizing control) or in the presence of genistein (50 μM) for PNU-282987 (0.1, 1, and 10 μM) added as indicated by the horizontal bars. b, c, and d, the concentration responses to ACh, GTS-21, and PNU-282987, respectively, in the presence or absence of genistein (50 μM). Each data point is $n = 3$ to 6. Summary of potency and maximum efficacy is summarized in Table 1.

PNU-120596 (Hurst et al., 2005). Among the agonists tested, the greatest effect was observed for GTS-21. In the absence of any modulator, this compound was a partial agonist, and in the presence of any one of the four modulators, GTS-21 turned out to be a very efficacious agonist. Analysis of the inhibition concentration-response curves to ACh revealed that both types of modulators affected the inhibition similarly. In this study, very little window current (overlap between inhibition and activation curves) to ACh alone (Fig. 9) was observed, consistent with previous observations (Briggs and McKenna, 1998). To our knowledge, effects of modulators on window currents have not been evaluated for any α7 PAM. Our studies demonstrate that type II PAMs, PNU-120596, and TQS, had in general larger effects on window currents than type I PAMs, genistein, and 5-HI.

In cultured rat hippocampal neurons, PNU-120956 activated desensitized rat α7 currents when studied electrophysiologically using whole-cell, patch-clamp recordings (Hurst et al., 2005). In addition to confirming this observation at human α7 currents, we found that PNU-120596 and TQS produced similar effects evoking current reactivation from desensitized α7 nAChRs in contrast to genistein and 5-HI that did not. The characteristics of this reactivated current (specifically its onset and weakly decaying nature) are similar to those of the secondary component described above (see Fig. 3 and *Distinct Profiles of nAChR PAMs: Types I and II*) suggesting that the same "activated" channel state is responsible for both. The mechanisms responsible for the induction of this activated state remain to be identified and require further investigation. Potential explanations could be that α7 nAChR modulators stabilize a new "desensitized-open" state, as in the case of the α7V274T mutant (Galzi et al., 1992; Briggs et al., 1999), or promote a shift in the equilibrium from a desensitized state to the "active" open state and stabilizing the receptor in the latter state as suggested for ivermectin (Krause et al., 1998).

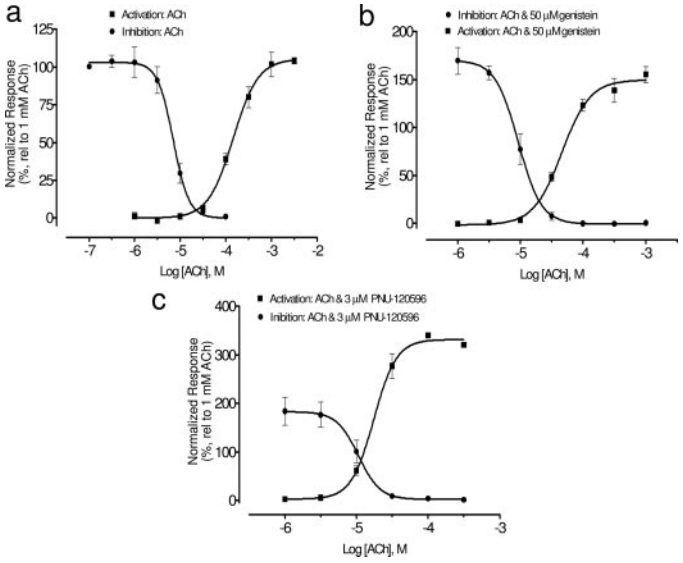

Fig. 9. Effects of α7 PAMs on window currents evoked by ACh. a, ACh activation and inhibition concentration response graphs without PAM. b and c, ACh activation and inhibition concentration graphs, respectively, in the presence of genistein (50 μM) or PNU-120596 (3 μM). Each data point is $n = 2$ to 6.

TABLE 2
Selectivity of PAMs at other human nAChR subtypes studied by Ca^{2+} imaging

	HEK $\alpha4\beta2$ pEC_{50} [Max-fold][a]	HEK $\alpha3\beta4$ pEC_{50} [Max-fold][a]
PNU-120596	N.A. [0.89 ± 0.11][b] ($n = 4$)	N.A. [0.80 ± 0.08][b] ($n = 4$)
TQS	5.3 ± 0.2 [0.27 ± 0.04] ($n = 4$)	N.A. [0.89 ± 0.07][c] ($n = 4$)
5-HI	3.4 ± 0.1 [0.21 ± 2.7] ($n = 6$)	2.9 ± 0.2 [0.28 ± 2.7] ($n = 4$)
Genistein	N.A. [0.60 ± 0.07][d] ($n = 6$)	N.A. [0.63 ± 0.10][d] ($n = 4$)

[a] Normalized to 3 to 10 μM nicotine.
[b] Effect at 30 μM.
[c] Effect at 10 μM.
[d] Effect at 100 μM.

Selectivity of α7 PAMs. Targeting PAMs rather than direct agonist could offer a potential advantage in terms of selectivity because PAM binding sites are likely distinct from agonist/competitive antagonist binding sites that show considerable homology among various nAChR and related ligand-gated ion channels of the cys-loop family. Determination of selectivity of PAMs will be important to avoid potential non-α7 nAChR interactions. For example, α3β4* receptors are thought to be involved in the control of bladder and cardiac function and α4β2* subunits in reinforcing effects of nicotine related to addiction (see review by Dani and Bertrand, 2007). In this study, we tested the effects of PNU-120596, TQS, genistein, and 5-HI on α4β2 and α3β4 subunits using Ca^{2+} flux measurements. None of these compounds evoked increases in the signals mediated by α4β2 and α3β4 nAChRs, indicating that they are selective PAMs for α7. Our observations are similar to those reported for PNU-120596 (tested only at 1 μM) in recordings from *X. laevis* oocytes expressing hα4β2, hα3β4, or hα9α10 nAChR (Hurst et al., 2005) and for genistein (at 10 μM) on ACh evoked currents in *X. laevis* oocytes expressing α4β2 and α3β4 subunits (Cho et al., 2005). In this study, significant inhibition of Ca^{2+} responses mediated by hα4β2 or hα3β4 subunits were noted for all compounds except PNU-120596 (see Table 2) at concentrations similar or slightly higher than those required for modulation of α7; the significance of which remains to be clarified. In addition to effects of 5-HI on nAChRs, this compound also positively modulates 5-HT_3 currents endogenously expressed in NCB-20 cells and N1E-115 neuroblastoma cells (van Hooft et al., 1997), limiting its usefulness as a tool compound.

In summary, this study shows that structurally distinct α7 PAMs can be divided into two types based on their effects on α7 currents. Type I PAMs—5-HI and genistein—predominantly affected the apparent peak current response, whereas type II PAMs—PNU-120596 and TQS—increased apparent peak current response and strongly evoked a secondary weakly decaying current. In general, type II but not type I PAMs could reactivate desensitized α7 currents and had greater effects shifting α7 agonist concentration-responses and on ACh window currents. The identification of distinct functional profiles of α7 PAMs and the reported demonstration of PAM efficacy in preclinical in vivo models of cognition provide basis for the development of novel therapeutics for CNS indications for which the α7 nAChR is considered a viable target.

References

Akiyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe S, Itoh N, Shibuya M, and Fukami Y (1987) Genistein, a specific inhibitor of tyrosine-specific protein kinases. *J Biol Chem* **262:**5592–5595.

Alkondon M, Braga MF, Pereira EF, Maelicke A, and Albuquerque EX (2000) Alpha7 nicotinic acetylcholine receptors and modulation of gabaergic synaptic transmission in the hippocampus. *Eur J Pharmacol* **393:**59–67.

Becker C, Comstock J, Michne WF, Murphy M, Philips E, Rosamond JD, and Simpson TR (2006), inventors; AstraZeneca, assignee. Positive modulators of nicotinic acetylcholine receptors. World Patent no. W02004098600(A1). 2006 Feb 1.

Blake RA, Broome MA, Liu X, Wu J, Gishizky M, Sun L, and Courtneidge SA (2000) SU6656, a selective src family kinase inhibitor, used to probe growth factor signaling. *Mol Cell Biol* **20:**9018–9027.

Bodnar AL, Cortes-Burgos LA, Cook KK, Dinh DM, Groppi VE, Hajos M, Higdon NR, Hoffmann WE, Hurst RS, Myers JK, et al. (2005) Discovery and structure-activity relationship of quinuclidine benzamides as agonists of alpha7 nicotinic acetylcholine receptors. *J Med Chem* **48:**905–908.

Breese CR, Adams C, Logel J, Drebing C, Rollins Y, Barnhart M, Sullivan B, Demasters BK, Freedman R, and Leonard S (1997) Comparison of the regional expression of nicotinic acetylcholine receptor alpha7 mRNA and [^{125}I]-alpha-bungarotoxin binding in human postmortem brain. *J Comp Neurol* **387:**385–398.

Briggs CA and McKenna DG (1998) Activation and inhibition of the human alpha7 nicotinic acetylcholine receptor by agonists. *Neuropharmacology* **37:**1095–1102.

Briggs CA, McKenna DG, Monteggia LM, Touma E, Roch JM, Arneric SP, Gopalakrishnan M, and Sullivan JP (1999) Gain of function mutation of the alpha7 nicotinic receptor: distinct pharmacology of the human alpha7V274T variant. *Eur J Pharmacol* **366:**301–308.

Briggs CA, McKenna DG, and Piattoni-Kaplan M (1995) Human alpha7 nicotinic acetylcholine receptor responses to novel ligands. *Neuropharmacology* **34:**583–590.

Broad LM, Zwart R, Pearson K, Lee M, Wallace L, Mc PG, Emkey R, Hollinshead S, Dell C, Baker R, et al. (2006) Identification and pharmacological profile of a new class of selective nicotinic acetylcholine receptor potentiators. *J Pharmacol Exp Ther* **318:**1108–1117.

Charpantier E, Wiesner A, Huh KH, Ogier R, Hoda JC, Allaman G, Raggenbass M, Feuerbach D, Bertrand D, and Fuhrer C (2005) Alpha7 neuronal nicotinic acetylcholine receptors are negatively regulated by tyrosine phosphorylation and Src-family kinases. *J Neurosci* **25:**9836–9849.

Chimienti F, Hogg RC, Plantard L, Lehmann C, Brakch N, Fischer J, Huber M, Bertrand D, and Hohl D (2003) Identification of SLURP-1 as an epidermal neuromodulator explains the clinical phenotype of Mal de Meleda. *Hum Mol Genet* **12:**3017–3024.

Cho CH, Song W, Leitzell K, Teo E, Meleth AD, Quick MW, and Lester RA (2005) Rapid upregulation of alpha7 nicotinic acetylcholine receptors by tyrosine dephosphorylation. *J Neurosci* **25:**3712–3723.

Conroy WG, Liu QS, Nai Q, Margiotta JF, and Berg DK (2003) Potentiation of α7-containing nicotinic acetylcholine receptors by select albumins. *Mol Pharmacol* **63:**419–428.

Couturier S, Bertrand D, Matter JM, Hernandez MC, Bertrand S, Millar N, Valera S, Barkas T, and Ballivet M (1990) A neuronal nicotinic acetylcholine receptor subunit (alpha 7) is developmentally regulated and forms a homo-oligomeric channel blocked by alpha-BTX. *Neuron* **5:**847–856.

Curzon P, Anderson DJ, Nikkel AL, Fox GB, Gopalakrishnan M, Decker MW, and Bitner RS (2006) Antisense knockdown of the rat alpha7 nicotinic acetylcholine receptor produces spatial memory impairment. *Neurosci Lett* **410:**15–19.

Dajas-Bailador F, and Wonnacott S (2004) Nicotinic acetylcholine receptors and the regulation of neuronal signalling. *Trends Pharmacol Sci* **25:**317–324.

Dani JA and Bertrand D (2007) Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. *Annu Rev Pharmacol Toxicol* **47:**699–729.

de Fiebre CM, Meyer EM, Henry JC, Muraskin SI, Kem WR, and Papke RL (1995) Characterization of a series of anabaseine-derived compounds reveals that the 3-(4)-dimethylaminocinnamylidine derivative is a selective agonist at neuronal nicotinic α7/[^{125}I]-α-bungarotoxin receptor subtypes. *Mol Pharmacol* **47:**164–171.

Dineley KT, Hogan D, Hernandez CM, Song W, Zheng H, and Sweatt JD (2005) Genetic deletion of the α7 nicotinic acetylcholine receptor decreases hippocampal Aβ accumulation but exacerbates hippocampal learning and memory deficits in APP_{SWE} mice. *Soc Neurosci Abstr* **33:**586.7

Felix R and Levin ED (1997) Nicotinic antagonist administration into the ventral hippocampus and spatial working memory in rats. *Neuroscience* **81:**1009–1017.

Frazier CJ, Rollins YD, Breese CR, Leonard S, Freedman R, and Dunwiddie TV (1998) Acetylcholine activates an alpha-bungarotoxin-sensitive nicotinic current in rat hippocampal interneurons, but not pyramidal cells. *J Neurosci* **18:**1187–1195.

Galzi JL, Devillers-Thiery A, Hussy N, Bertrand S, Changeux JP, and Bertrand D (1992) Mutations in the channel domain of a neuronal nicotinic receptor convert ion selectivity from cationic to anionic. *Nature* 359:500–505.

Gotti C, Riganti L, Vailati S, and Clementi F (2006) Brain neuronal nicotinic receptors as new targets for drug discovery. *Curr Pharm Des* **12:**407–428.

Hajós M, Hurst RS, Hoffmann WE, Krause M, Wall TM, Higdon NR, and Groppi VE (2005) The selective alpha7 nicotinic acetylcholine receptor agonist PNU-282987 [*N*-[(3*R*)-1-azabicyclo[2.2.2]oct-3-yl]-4-chlorobenzamide hydrochloride] enhances GABAergic synaptic activity in brain slices and restores auditory gating deficits in anesthetized rats. *J Pharmacol Exp Ther* **312:**1213–1222.

Hanke JH, Gardner JP, Dow RL, Changelian PS, Brissette WH, Weringer EJ, Pollok BA, and Connelly PA (1996) Discovery of a novel, potent, and Src family-selective tyrosine kinase inhibitor. Study of Lck- and FynT-dependent T cell activation. *J Biol Chem* **271:**695–701.

Hevers W and Luddens H (1998) The diversity of $GABA_A$ receptors. Pharmacological and electrophysiological properties of $GABA_A$ channel subtypes. *Mol Neurobiol* **18:**35–86.

Hurst RS, Hajos M, Raggenbass M, Wall TM, Higdon NR, Lawson JA, Rutherford-Root KL, Berkenpas MB, Hoffmann WE, Piotrowski DW, et al. (2005) A novel positive allosteric modulator of the alpha7 neuronal nicotinic acetylcholine receptor: in vitro and in vivo characterization. *J Neurosci* **25:**4396–4405.

Keller JJ, Keller AB, Bowers BJ, and Wehner JM (2005) Performance of alpha7 nicotinic receptor null mutants is impaired in appetitive learning measured in a signaled nose poke task. *Behav Brain Res* **162:**143–152.

Krause RM, Buisson B, Bertrand S, Corringer PJ, Galzi JL, Changeux JP, and Bertrand D (1998) Ivermectin: a positive allosteric effector of the α7 neuronal nicotinic acetylcholine receptor. *Mol Pharmacol* **53:**283–294.

Levin ED and Rezvani AH (2002) Nicotinic treatment for cognitive dysfunction. *Curr Drug Targets CNS Neurol Disord* **1:**423–431.

Mok MHS and Kew JN (2006) Excitation of rat hippocampal interneurons via modulation of endogenous agonist activity at the alpha7 nicotinic ACh receptor. *J Physiol* **574:**699–710.

Ng HJ, Whittemore ER, Tran MB, Hogenkamp DJ, Broide RS, Johnstone TB, Zheng L, Stevens KE, and Gee KW (2007) Nootropic alpha7 nicotinic receptor allosteric modulator derived from $GABA_A$ receptor modulators. *Proc Natl Acad Sci U S A* **104:**8059–8064.

Paterson D and Nordberg A (2000) Neuronal nicotinic receptors in the human brain. *Prog Neurobiol* **61:**75–111.

Rubboli F, Court JA, Sala C, Morris C, Chini B, Perry E, and Clementi F (1994) Distribution of nicotinic receptors in the human hippocampus and thalamus. *Eur J Neurosci* **6:**1596–1604.

Samochocki M, Hoffle A, Fehrenbacher A, Jostock R, Ludwig J, Christner C, Radina M, Zerlin M, Ullmer C, Pereira EF, et al. (2003) Galantamine is an allosterically potentiating ligand of neuronal nicotinic but not of muscarinic acetylcholine receptors. *J Pharmacol Exp Ther* **305:**1024–1036.

Trumbull JD, Maslana ES, McKenna DG, Nemcek TA, Niforatos W, Pan JY, Parihar AS, Shieh CC, Wilkins JA, Briggs CA, et al. (2003) High throughput electrophysiology using a fully automated, multiplexed recording system. *Receptors Channels* **9:**19–28.

van Hooft JA, van der HE, and Vijverberg HP (1997) Allosteric potentiation of the 5-HT_3 receptor-mediated ion current in N1E-115 neuroblastoma cells by 5-hydroxyindole and analogues. *Neuropharmacology* **36:**649–653.

Van Kampen M, Selbach K, Schneider R, Schiegel E, Boess F, and Schreiber R (2004) AR-R 17779 improves social recognition in rats by activation of nicotinic alpha7 receptors. *Psychopharmacology (Berl)* **172:**375–383.

Wehner JM, Keller JJ, Keller AB, Picciotto MR, Paylor R, Booker TK, Beaudet A, Heinemann SF, and Balogh SA (2004) Role of neuronal nicotinic receptors in the effects of nicotine and ethanol on contextual fear conditioning. *Neuroscience* **129:**11–24.

Wevers A, Jeske A, Lobron C, Birtsch C, Heinemann S, Maelicke A, Schroder R, and Schroder H (1994) Cellular distribution of nicotinic acetylcholine receptor subunit mRNAs in the human cerebral cortex as revealed by non-isotopic in situ hybridization. *Brain Res Mol Brain Res* **25:**122–128.

Wishka DG, Walker DP, Yates KM, Reitz SC, Jia S, Myers JK, Olson KL, Jacobsen EJ, Wolfe ML, Groppi VE, et al. (2006) Discovery of *N*-[(3*R*)-1-Azabicyclo[2.2.2]oct-3-yl]furo[2,3-c]pyridine-5-carboxamide, an agonist of the alpha7 nicotinic acetylcholine receptor, for the potential treatment of cognitive deficits in schizophrenia: synthesis and structure-activity relationship. *J Med Chem* **49:**4425–4436.

Yanagihara N, Tachikawa E, Izumi F, Yasugawa S, Yamamoto H, and Miyamoto E (1991) Staurosporine: an effective inhibitor for Ca^{2+}/calmodulin-dependent protein kinase II. *J Neurochem* **56:**294–298.

Zakar T, Mijovic JE, Bhardwaj D, and Olson DM (1999) Tyrosine kinase inhibitors block the glucocorticoid stimulation of prostaglandin endoperoxide H synthase expression in amnion cells. *Can J Physiol Pharmacol* **77:**138–142.

Zbarsky V, Thomas J, and Greenfield S (2004) Bioactivity of a peptide derived from acetylcholinesterase: involvement of an ivermectin-sensitive site on the alpha7 nicotinic receptor. *Neurobiol Dis* **16:**283–289.

Zwart R, De Filippi G, Broad LM, McPhie GI, Pearson KH, Baldwinson T, and Sher E (2002) 5-Hydroxyindole potentiates human alpha 7 nicotinic receptor-mediated responses and enhances acetylcholine-induced glutamate release in cerebellar slices. *Neuropharmacology* **43:**374–384.

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