Therapeutic Implications of a Selective $\alpha_7$ Nicotinic Receptor Abnormality in Schizophrenia

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Abstract: A convergence of preclinical pharmacology, and human autopsy and genetic data support the existence of reduced expression and function of the $\alpha_7$ nicotinic receptor in patients with schizophrenia. The $\alpha_7$ nicotinic receptor is a member of a family of ligand-gated ion channels. The $\alpha_7$ nicotinic receptor may play an essential role in auditory sensory gating and voluntary smooth pursuit eye movements, two psychophysiological functions that are abnormal in patients with schizophrenia and closely related unaffected biological relatives. Diminished expression or function of the $\alpha_7$ nicotinic receptor in schizophrenia has stimulated consideration of selective full or partial $\alpha_7$ nicotinic receptor agonists as possible therapeutic interventions for this disorder. Further, the availability of positive allosteric modulators of nicotinic receptors that can improve the efficiency of transduction of the acetylcholine signal and prevent the rapid desensitization of the receptor should encourage these novel treatment approaches (e.g., galantamine).

Nicotinic Acetylcholine Receptors: Structural and Functional Considerations

Nicotinic acetylcholine receptors (nAChRs) are members of a large family of ligand-gated ion channels, which include receptors for GABA, glycine and serotonin, that mediate “fast” synaptic transmission or signaling events within a millisecond timeframe (1-3). Members of this receptor family are pentameric proteins comprised of five polypeptide subunits, each of which is derived from a common ancestral protein. Although at least 16 genetically distinct, albeit related, types of nicotinic acetylcholine receptor subunits have been cloned from several species (referred to as $\alpha_1$-$\alpha_9$, $\beta_1$-$\beta_4$, $\gamma$, $\delta$, $\epsilon$), they all share a common motif with a large extracellular N-terminal hydrophilic domain, three transmembranous hydrophobic domains (M1-M3), an intracellular loop, and a final C-terminal transmembranous segment (M4). The extracellular domains have one or more glycosylation sites, and the intracellular loop possesses consensus sequences of amino acids that serve as substrates for enzymatic phosphorylation. The M2 transmembranous domains from each of the five polypeptide receptor subunits align themselves in a manner to surround a potential pore or channel that traverses the lipid bilayer.

The transient assumption of the open configuration of this channel, or activation of the receptor, allowing cationic conductance, is more likely to occur as a result of the binding of acetylcholine. Further, allosteric modulatory sites distinct from the agonist recognition site for acetylcholine exist on the receptor; ligands binding to these sites influence the likelihood that acetylcholine will be effective in promoting the transition from the closed configuration of the channel to an open one (1-4). Subsequent to the binding of acetylcholine, the channels will also transition into a desensitized or refractory state, which neither permits cationic conductance nor is sensitive to activation by acetylcholine. Allosteric modulatory ligands may also influence the kinetics of transitions into the desensitized state and the du-
ration of this refractory condition. Thus, in diseases associated with diminished expression of functional nAChRs, or genetically altered receptors that are less responsive to agonist, positive allosteric modulators may improve faulty signal transduction by increasing the frequency and lifetime of channel opening, and preserving the reduced number of functional receptors in a responsive state.

As demonstrated by in vitro expression, α7 receptor subunits can assemble with each other to form a functional homopentameric receptor that can be competitively antagonized by α-bungarotoxin (5). Further, the α7 nicotinic receptor has unique channel characteristics, including relatively high calcium ion permeability and rapid desensitization, which is reflected in a characteristic electrophysiological tracing referred to as type 1A (4). Another very unique feature of the α7 nicotinic receptor is the fact that choline, which is both a precursor and hydrolytic split product of acetylcholine, is a full agonist (4, 6–8). Through the local action of acetylcholinesterase, choline can be generated very rapidly within the local area of cholinergic synapses. The rapid kinetics with which choline, a selective α7 nicotinic receptor agonist, can stimulate and desensitize this receptor suggests that its formation may be another important mechanism of local regulation of α7 nicotinic receptor-mediated neurotransmission.

α7 Nicotinic receptors located presynaptically play an important role in the calcium ion-dependent regulation of release of a variety of neurotransmitters, whereas their postsynaptic location on GABAergic inhibitory interneurons, especially in hippocampus, supports a significant role in the regulation of inhibitory tone (5). The α7 nicotinic receptor may also play a critical role in brain development, regulating the differentiation of neurons and the formation of appropriate afferent cholinergic connections from such areas as basal forebrain (5).

Figure 1. Panels A and B depict representations of the nicotinic cholinergic receptor in the closed and open configurations, respectively. Each panel views the ligand-gated channel receptor while looking at the membrane surface facing the extracellular space and a cross-sectional slice of the channel embedded in the lipid bilayer. The binding of acetylcholine increases the likelihood that the channel will transition from the closed to the open configuration. Allosteric modulators bind to sites on the receptor complex that are distinct from the binding site for acetylcholine. The binding of allosteric modulators alters the binding characteristics of agonists and other channel ligands, the efficiency of coupling between agonist binding and channel opening, and the length of time the channel will remain in the closed, open, and refractory transition states. Galantamine is a positive allosteric modulator of acetylcholine. Galantamine improves the efficiency of coupling between the binding of acetylcholine and the assumption of the open or “activated” channel configuration. Further, the binding of galantamine decreases the likelihood that the channel will rapidly desensitize subsequent to the binding of acetylcholine. This figure is reprinted with the permission of Cynthia J. Gordon, Managing Editor of Central Nervous System/Long Term Care.
Nicotinic Cholinergic Receptor Abnormalities in Schizophrenia: Pathophysiological Considerations

The sensory flooding and inability to filter out relevant environmental stimuli from background noise experienced by patients with schizophrenia may relate to a fundamental defect in sensory gating (9-11). In human subjects, sensory gating may be quantified by the P50 wave, which is an evoked potential measured in response to each of a pair of auditory stimuli presented 500 milliseconds apart. The first or conditioned stimulus activates the inhibitory gating mechanism such that the amplitude of the second evoked potential is significantly reduced. In schizophrenia patients, this blunted response to the second stimulus of the pair is not observed, verifying a sensory gating defect or impairment of sensory inhibition in response to paired auditory stimuli. The sensory gating defect is not normalized by conventional antipsychotic medication, suggesting that its mechanism may not directly involve abnormal dopaminergic neurotransmission. Further, approximately 50 percent of the first-degree biological relatives of index patients with schizophrenia, most of whom are unaffected with schizophrenia, show this abnormality of sensory gating; thus, this trait may be inherited in an autosomal dominant mode. Also, inheritance of this trait may be a “necessary,” but not “sufficient” condition, for expression of overt schizophrenia. Finally, if, as is believed, this fundamental defect in signal processing underlies many of the attentional and cognitive abnormalities in schizophrenia, which contribute to functional disability and morbidity, it should be a primary target of the current pharmacotherapy of this disorder.

As discussed, the α7 subunit can form a functional homopentameric nicotinic receptor with unique electrophysiological and binding characteristics, including the relatively selective low-affinity binding of α-bungarotoxin, a snake venom (12, 13). In humans, α-bungarotoxin-sensitive cholinergic neurons within specific areas of the hippocampus and ventral lateral geniculate may participate in the normal inhibitory gating of the P50 evoked potential in response to repeated auditory stimuli and smooth pursuit eye movement performance, respectively. Interestingly, impairment of both the sensory inhibition of the auditory evoked response and smooth pursuit eye movement performance occur commonly in index patients with schizophrenia and their biological relatives (11, 14).

Smooth pursuit eye movement abnormalities in schizophrenia patients and unaffected first-degree biological relatives are highly replicable findings (14). Retinal motion or the “slippage” of the image of a slowly moving target on the retina initiates smooth pursuit, which is processed in the medial temporal region. The maintenance of normal smooth pursuit depends on “predictive” smooth pursuit eye movements and occasional rapid saccadic movements in order to catch up with the target. Medial superior temporal and posterior parietal regions and the frontal eye fields integrate the information necessary for the maintenance of normal smooth pursuit. Schizophrenia patients show abnormalities of both initiation of smooth pursuit and predictive smooth pursuit, which may relate to liability for the deficit syndrome and psychosis, respectively (14). Nicotine’s effects, administered as a pulse within seconds via a nasal spray, on specific measures of smooth pursuit and visual attention were assessed in 29 patients with schizophrenia and 26 age-matched control subjects. Irrespective of smoking status, the rapid pulse of nicotine improved eye acceleration during initiation of smooth pursuit in the group of patients with schizophrenia; it did not improve this measure in the normal subjects. Further, irrespective of subject group or smoking status, nicotine improved “closed loop pursuit gain” or the extent to which eye velocity matches target velocity. There was no effect of nicotine observed on vigilance or attention, as assessed with a visually guided saccadic eye movement task and continuous performance task in any of the groups (14). The positive effect of nicotine on a measure of smooth pursuit in schizophrenia suggested that it improved the processing of retinal motion information and the integration of sensory information with the motor response. Further, this effect was apparently independent of a beneficial effect on vigilance and attention. Interestingly, schizophrenia patients and control subjects who smoke differed from each other on a formal measure of nicotine dependence; the patients were rated as more dependent. Whereas higher dependence was associated with better closed loop gain in normal controls,
higher nicotine dependence was associated with poorer performance on this measure in schizophrenia patients. These data are consistent with differences in sensitivity to nicotine and nAChRs in patients and controls.

The ability of orally administered nicotine to improve abnormal sensory gating of the P50 auditory evoked potential was studied in six unaffected first or second degree nonsmoking relatives of patients with schizophrenia, diagnosed according to DSM-III-R criteria (15). The severity of the sensory gating deficit in these subjects did not differ significantly from that observed in patients with schizophrenia. The psychophysiological deficit in these unaffected relatives was not confounded by the psychosocial dysfunction, brain damage, and medication effects seen in patients with schizophrenia. Treatment with nicotine gum transiently improved the gating (15). Thus, nicotine is able to transiently normalize a familial psychophysiological deficit; unfortunately, nAChRs rapidly desensitize upon exposure to agonist.

The auditory P50 gating deficit in schizophrenia was transiently normalized in 10 patients, who were allowed to smoke freely after overnight abstinence (16). After obtaining two baseline recordings, two additional EEG recordings were obtained within 5 minutes and 15 minutes after smoking; the patients and an equal number of age-matched controls smoked two to four cigarettes over a period of 30 to 45 minutes. This significant effect of cigarette smoking on the "P50 ratio" in the schizophrenic patients was observed in the first postsmoking trial. By the second postsmoking trial, the P50 ratio of the schizophrenic patients was returning to the abnormally high baseline value. Thus, the normalizing effect of nicotine on sensory gating abnormalities in schizophrenia was of a brief duration. Importantly, there were no significant effects of nicotine on the amplitude or latency of the evoked response to the conditioning stimulus in either the patient or control groups. Therefore, nicotine's primary effect was on the gating deficit in schizophrenia, rather than on the perception and processing of the auditory stimulus. Interestingly, the overnight abstinence from smoking affected the P50 ratio of the 10 normal smokers such that their initial baseline measurement was significantly higher than a reference group of 43 normal subjects (16). Thus, the sensitivity of nicotinic receptors, especially those involved in auditory sensory gating, may fluctuate rapidly in normal subjects.

The P50 wave recorded from the scalp surface reflects the burst of excitatory activity in the network of highly interconnected hippocampal CA3 pyramidal cell neurons (11). In addition to activating pyramidal cells via excitatory projections from the entorhinal cortex and dentate gyrus, the first auditory stimulus also activates a cholinergic input from the basal forebrain. Ultimately, it is this cholinergic input transduced by $\alpha_7$ nicotinic receptors located on the surface of inhibitory interneurons that leads to a gating of the response to the second auditory stimulus presented 500 milliseconds later. Conceivably, a failure of this fundamental gating mechanism in hippocampus, especially in the highly interconnected excitatory CA3 pyramidal neurons, could contribute to, or be manifest as, the loosening of associations, in addition to the experience of sensory flooding, of schizophrenia.

Nicotine was recently shown to improve finger tapping rate in schizophrenia patients medicated with stable regimens of atypical antipsychotic medications; finger tapping rate is a measure that reflects central processing speed (17). Essentially, finger tapping rate was compared between 17 inpatients with schizophrenia who smoked at least 20 cigarettes per day and 17 inpatients who did not smoke. The significantly higher rates of finger tapping among smokers for both the right and left hands did not appear to be an artifact of differences between the groups in severity of extrapyramidal movements or tardive dyskinesia, dose of atypical antipsychotic medication, or smoking-induced changes in the metabolism of the atypical antipsychotic medications (17). The data suggest that nicotine can have salutary therapeutic effects on a complex gross measure of central processing in patients with schizophrenia. However, the relevance of this improvement to a specific subpopulation of nicotinic receptors is not known.
Nicotinic Cholinergic Receptor Abnormalities in Schizophrenia: Anatomic Pathology

The percentages of the area of various 10-fold magnified microscopic fields labeled by $^{125}\text{I}$- $\alpha$-bungarotoxin in specific hippocampal regions (especially dentate gyrus, CA3 region of Ammon’s horn, and CA1 region) were compared in autopsied brains obtained from eight patients with schizophrenia and eight age- and sex-matched nonschizophrenic control subjects (18). In the dentate hilus and CA3 region, the schizophrenic patients showed a significant reduction in the percentage of area labeled with $^{125}\text{I}$- $\alpha$-bungarotoxin, whereas the groups did not differ in the CA1 region. The reduction of labeling was most pronounced in the dentate gyrus; 7 of the patients had values for the density of microscopic fields labeled in this region that were below the lowest value of the nonschizophrenic controls. In addition to the reduction in the number of labeled cells among patients with schizophrenia, the individual cells themselves appeared less heavily labeled without the same extension of the labeling to the dendritic processes, compared with controls (18). The dentate hilus and CA3 region receive a dense cholinergic innervation from the medial septal nucleus. Thus, the reduction in labeling with $^{125}\text{I}$- $\alpha$-bungarotoxin, a relatively selective marker of the low-affinity $\alpha_7$ nicotinic receptor, in patients with schizophrenia supports the existence of a selective abnormality of signal transduction in at least a subpopulation of patients with schizophrenia. Further, the localization of $\alpha_7$ nicotinic receptors to GABAergic inhibitory interneurons contributes to converging evidence for reduced number and/or function of these inhibitory interneurons.

The reticular nucleus of the thalamus receives a dense cholinergic innervation from the medial septal nucleus. Thus, the reduction in labeling with $^{125}\text{I}$- $\alpha$-bungarotoxin, a ligand that is selective for the $\alpha_7$ nicotinic receptor subunit antibody (20). Specifically, solubilized fractions of membrane protein receptors were prepared from autopsied brains of eight patients with chronic schizophrenia and eight age-matched nonpsychiatric controls. The identity of the single band as containing the $\alpha_7$ nicotinic receptor subunit was confirmed by the specific binding of $^{125}\text{I}$- $\alpha$-bungarotoxin, a ligand that is selective for the $\alpha_7$ nicotinic receptor subunit. A comparison of the patient and control samples showed a 40 percent reduction in the amount of $\alpha_7$ subunit in frontal cortices obtained from the patients with schizophrenia, compared to the controls, whereas the amount of $\alpha_7$ subunit between the two groups did not differ in parietal cortex. A regionally selective reduction in expression of functional $\alpha_7$-containing nicotinic receptors in frontal cortex may relate to some of the symptoms and disordered sensory perceptions of this illness (19). Further, there were even more profound reductions of $^{125}\text{I}$- $\alpha$-bungarotoxin binding in reticular nuclei from an autopsied series of 14 patients with dementia with Lewy bodies (DLB). DLB is characterized by progressive cognitive decline, visual hallucinations, and transient alterations of awareness and attention. Perhaps these symptoms could reflect a failure to activate GABAergic inhibitory mechanisms because of loss of these inhibitory interneurons, or defective or diminished transduction of the acetylcholine signal by $\alpha_7$ nicotinic receptors located on their surface.

A single 42 kDa band was identified after Western blots of Triton X-100 extracts of autopsied human brain homogenates, prepared in the presence of protease inhibitors, were incubated separately in the presence of mouse monoclonal anti-nAChR $\alpha_7$ subunit antibody and goat polyclonal anti-nAChR $\alpha_7$ subunit antibody. Specifically, solubilized fractions of membrane protein receptors were prepared from autopsied brains of eight patients with chronic schizophrenia and eight age-matched nonpsychiatric controls. The identity of the single band as containing the $\alpha_7$ nicotinic receptor subunit was confirmed by the specific binding of $^{125}\text{I}$- $\alpha$-bungarotoxin, a ligand that is selective for the $\alpha_7$ nicotinic receptor subunit. A comparison of the patient and control samples showed a 40 percent reduction in the amount of $\alpha_7$ subunit in frontal cortices obtained from the patients with schizophrenia, compared to the controls, whereas the amount of $\alpha_7$ subunit between the two groups did not differ in parietal cortex. A regionally selective reduction in expression of functional $\alpha_7$-containing nicotinic receptors in frontal cortex may relate to some of the symptoms and disordered sensory perceptions of this illness (19). Further, there were even more profound reductions of $^{125}\text{I}$- $\alpha$-bungarotoxin binding in reticular nuclei from an autopsied series of 14 patients with dementia with Lewy bodies (DLB). DLB is characterized by progressive cognitive decline, visual hallucinations, and transient alterations of awareness and attention. Perhaps these symptoms could reflect a failure to activate GABAergic inhibitory mechanisms because of loss of these inhibitory interneurons, or defective or diminished transduction of the acetylcholine signal by $\alpha_7$ nicotinic receptors located on their surface.
symptoms of “hypofrontality” observed in patients with schizophrenia (20).

Quantitative autoradiography was used to evaluate the laminar distribution of $^{[125I]}\alpha$-bungarotoxin binding in orbitofrontal, posterior temporal, and anterior cingulate cortices obtained from autopsied brains of 12 schizophrenia patients and 14 age-matched control subjects, and compare the density of binding between the groups (21). Because of the high prevalence of smoking in schizophrenia (as high as 70% — 80% compared to 30% in the general population), comparisons were also made with a subgroup of control subjects who were smokers at the time of death. Tissue was not available in all of the cortical areas for all of the patients. For both the control and patient groups, the binding of $^{[125I]}\alpha$-bungarotoxin was distributed uniformly across all cortical layers in all three areas. Further, when compared with an age-matched subgroup of smoking controls ($n=7$), the density of $^{[125I]}\alpha$-bungarotoxin binding of schizophrenia patients ($n=10$) was reduced in the anterior cingulate cortex by about 54 percent (21). The selective reduction of $^{[125I]}\alpha$-bungarotoxin binding found in this study suggests that, in spite of its widespread and homogeneous distribution, expression of the functional homopentameric $\alpha_7$ nicotinic receptor may be selectively reduced in specific areas of the brain in schizophrenia.

Quantitative histological investigation of the density of neurons in the prefrontal area (Brodmann’s area 10) and anterior cingulate (Brodmann’s area 24) were performed in postmortem tissues from control subjects and patients with schizophrenia distinguished according to those without and with a superimposed mood disturbance (22). In the schizophrenia patients, the number of smaller interneurons was reduced in layer II of the anterior cingulate and prefrontal cortices, and the number of pyramidal neurons in layer V of the prefrontal cortex was increased, when mean cell counts were adjusted for the possible confounding effects of age, duration of fixation, hypoxia index, and postmortem interval. Glial cell counts did not differ significantly between the groups. A reduction of small inhibitory interneurons that use GABA should result in diminished inhibitory tone in local circuits that, in turn, influence the outputs of these areas to distant cortical and subcortical sites (22). Further, these histological data are consistent with a possible interpretation of the data on $^{[125I]}\alpha$-bungarotoxin binding (i.e., actual neuronal cell loss) noted in the previous paragraph. Interestingly, hypoxic events and other types of insults that can result in neuronal cell loss can do so in the perinatal period unaccompanied by gliosis. Further, basket neurons, which are a type of small GABAergic inhibitory interneuron, are still somewhat immature at birth, especially those in layer II; thus, they may be more vulnerable to hypoxic and other environmental insults. Although most children with perinatal insults do not manifest schizophrenia as adults, the selective loss of these neurons in local circuits may contribute to increased risk of overt manifestation of illness, especially if other conditions are satisfied.

$\alpha_7$ Nicotinic Cholinergic Receptor Abnormalities in Schizophrenia: Animal Models

Using inbred mouse strains, an abnormality of sensory inhibition was shown to be a stably inherited trait, whose severity correlated inversely with the density of $\alpha$-bungarotoxin binding sites in hippocampus (23). Essentially, sensory inhibition is demonstrated in the electrophysiological recordings of hippocampal CA3 pyramidal cell neurons to the second of a pair of auditory stimuli presented 500 milliseconds apart. In the mouse, a complex is reliably recorded with a maximally negative deflection referred to as N40 occurring between 20 and 60 milliseconds after an auditory stimulus; the N40 wave is preceded by a positive deflection referred to as P20. Ordinarily, the response to the second of the paired stimuli, referred to as the test stimulus, is blunted and the extent of this sensory inhibition is expressed as the ratio of the amplitudes of the responses to the test stimulus versus the “conditioned” stimulus or first stimulus of the pair. Sensory inhibition of the evoked P20-N40 complex to the second of the paired auditory stimuli is dependent on the intactness of the septal-hippocampal cholinergic projections. The lesioning of the fimbria-fornix, which contains this cholinergic projection, disrupts sensory inhibition. This is further evidence of an important cholinergic regulatory influence on sensory inhibition. The
DBA/2 inbred mouse strain resembles patients with schizophrenia in terms of the failure to blunt or gate the electrophysiological response to the test stimulus. This mouse strain showed normalization of sensory inhibition in response to nicotinic interventions; moreover, the normalized response to nicotinic interventions was blocked by α-bungarotoxin, a selective α7 nicotinic receptor antagonist, but not by mecamylamine, a selective antagonist of α4β2 nicotinic receptors.

Importantly, a major theoretical limitation to the development of nicotinic agonist interventions, such as nicotine itself, for the treatment of impaired sensory gating in schizophrenia and other disorders is the rapid desensitization of the nicotinic receptor to this effect (24). Thus, the ability of GTS-21 and two other related anabaseine compounds, which are partial α7 nicotinic receptor agonists, to normalize sensory inhibition in DBA/2 mice without causing rapid desensitization is very encouraging. Specifically, the decrease in amplitude of the auditory-evoked P20-N40 complex in DBA/2 mice to the test stimulus after treatment with GTS-21 persisted for as long as 20 minutes after an initial injection, and the magnitude and duration of response was again observable upon a repeated injection 40 minutes later. Again, the beneficial effect of GTS-21 on normalization of sensory inhibition was antagonized by α-bungarotoxin, but not mecamylamine, consistent with its selective interaction with the α7 nicotinic receptor (24). Further, from a theoretical perspective, a partial α7 nicotinic receptor agonist may possess a lower liability for causing nicotine-induced seizures than full agonists.

The DBA/2 strain shows both a profound deficit in sensory inhibition and a markedly decreased density of hippocampal α7 nicotinic receptors (23). Thus, this mouse strain may serve as an animal model of an important sensory gating abnormality of schizophrenia, which may be a “necessary” condition for at least some presentations of overt illness. A viable medication strategy to address this possible “fundamental” defect would be one that can be administered orally without loss of efficacy upon chronic administration (25). To this end, the ability of intragastrically administered dimethoxybenzylidine anabaseine (DMXB-A, also known as GTS-21), via an esophageal tube, to suppress the amplitude of the hippocampal P20-N40 wave to the second of a pair of auditory stimuli, was studied in anesthetized DBA/2 mice (25). DMXB-A was shown to be efficacious after intragastric administration; however, the dose-response relation was U-shaped. The 10 mg/kg dose produced a significant effect, whereas the 1, 3.3, and 33 mg/kg doses did not. Further, the effect resulted from a selective decrease in the amplitude of the response to the second or test stimulus. Thus, the effect was on sensory gating, rather than a nonspecific effect on hippocampal excitability in general. The positive effect of intragastrically administered DMXB-A on sensory inhibition in DBA/2 mice was antagonized by the intraventricular administration of α-bungarotoxin, but not mecamylamine. Thus, the positive effect is mediated by stimulation of α7 nicotinic receptors. These data support development of an oral partial α7 nicotinic receptor agonist for schizophrenia; however, there was a suggestion of possible desensitization to the positive therapeutic effect (25).

Curiously, although conventional antipsychotic medications do not improve auditory sensory gating, amphetamine administration can simulate an auditory processing deficit in humans and rodents (26). In unanesthetized male Sprague-Dawley rats, the intraperitoneal administration of 1.83 mg/kg of d-amphetamine sulfate produces the loss of sensory gating with a minimum of interfering stereotypies. Using this standard dose of amphetamine to mimic the sensory gating defect pharmacologically, subcutaneously administered nicotine bitartrate lowered the TC ratio to control levels at doses that did not affect the evoked potentials or auditory gating by themselves. This normalizing effect of nicotine on the disruptive effects of amphetamine was mediated by nicotinic receptors as it in turn was blocked by the intracerebroventricular administration of d-tubocurarine (400 μM). Importantly, amphetamine’s most profound effect was to decrease the amplitude of response to the conditioned stimulus, the first stimulus of the pair of auditory stimuli, whereas nicotine modulated the ratio by a predominant effect on reducing the amplitude of response to the test stimulus (26). Nicotine was also able to suppress amphetamine-induced increases of locomotor activity. However, differences in the time course for nicotine’s effect on locomotion (early) and disrupted...
sensory gating (later) and the fact that the sensory gating abnormality was present during suppression of amphetamine-induced locomotion suggest that these effects of nicotine are mediated by different nicotinic receptors.

The abnormal prepulse inhibition (PPI) of the acoustic startle response in rats and mice is a model of the deficient sensorimotor gating in schizophrenic patients (27). In fact, the ability of a test compound to normalize impaired PPI of the acoustic startle response may be predictive of its "antipsychotic" efficacy (28). The startle response is an abrupt movement of the whole animal, usually detected in an automated fashion by the electrical transduction of the force of vertical displacement on a platform, in response to the unpredictable presentation of a loud noise. The intensity of the startle response to the startle stimulus can be attenuated if the startle stimulus is preceded with a pulse of noise that is significantly less loud and of briefer duration than the startle stimulus itself. Inbred mouse strains have been identified that show reliable impairment of the PPI of the acoustic startle response; for example, the DBA/2J (D2) mouse strain shows diminished PPI (29). Intraperitoneally administered clozapine and risperidone have been shown to improve the PPI of the acoustic startle response in male DBA/2J mice, whereas a variety of intraperitoneally administered doses of the selective partial (GTS-21; 0, 1, 3, and 10 mg/kg) and full (AR-R17779; (-)-spiro[1-azabicyclo[2.2.2]octane-3,5’-oxazolidin]-2’-one; 0, 1, 3 and 10 mg/kg) \( \alpha_7 \) nicotinic receptor agonists were without significant effect on the PPI of the acoustic startle response (27). These selective nicotinic receptor agonist interventions were studied because of their ability to improve sensory inhibition of the evoked P20-N40 wave to a brief stimulus duration in the DBA strain, which has a reduced density of hippocampal \( \alpha_7 \) nicotinic receptor binding sites (24). Clearly, there are significant differences in the preclinical paradigms used to assess the sensory inhibition of the hippocampal P20-N40 wave and the prepulse inhibition of the acoustic startle response. For example, the former reflects an evoked electrophysiological response recorded from the hippocampal CA3 region of anesthetized mice after a brief latency following the presentation of the test stimulus, whereas the startle response is an integrated whole animal motor response. These data suggest that the two procedures reflect neurochemically distinct dimensions (27).

\( \alpha_7 \) Nicotinic Cholinergic Receptor Abnormalities in Schizophrenia: Human Genetic Evidence

The P50 auditory-evoked potential abnormality is a trait whose inheritance appears to follow an autosomal dominant mode (30). The segregation of this trait among patients with schizophrenia and its ability to discriminate between schizophrenia patients and normals is both dramatic and robust. For example, 91% of 36 unrelated patients with schizophrenia had a ratio of \( \geq 0.50 \) for the value of the amplitude of the P50 response to the second stimulus compared with the first, whereas only 6% of 43 unrelated normal controls had a ratio of 0.50 or greater. When the genetic transmission of this trait was studied in nine multiplex schizophrenic pedigrees (i.e., nuclear families containing at least two affected individuals), impairment of sensory inhibition was usually found in one parent and about half of the siblings, many of whom were clinically unaffected with schizophrenia. Thus, this trait has been referred to as an endophenotype, which may represent an “alternative” expression of a latent trait that can manifest itself as overt schizophrenia in some unfortunate individuals. In this model, manifestation of overt schizophrenia arises if the trait combines or interacts with other pathogenic elements (31). The preclinical and autopsy data implicating diminished expression and function of the \( \alpha_7 \)-nicotinic receptor underlying this sensory gating abnormality and the chromosomal localization of the gene for this receptor to 15q13-14 led to a linkage study with informative markers at the \( \alpha_7 \)-nicotinic receptor gene locus (30). Assuming an autosomal dominant mode for transmission of the P50 sensory gating abnormality, a genome-wide linkage analysis was performed of the P50 abnormality and 542 highly polymorphic markers, spaced approximately 10-centimorgans apart from each other, in the nine multiplex schizophrenic pedigrees (30). The pairwise lod score linkage analysis revealed a significant likelihood of linkage of the P50 abnormality with D15S1360, a genetic marker that is within 120 kb of the coding region of the gene.
for the $\alpha_7$-nicotinic receptor subunit (30, 32). These data support a possible causal association between the P50 auditory sensory abnormality and the gene for the $\alpha_7$-nicotinic receptor subtype in schizophrenia.

Very recently, data were presented supporting an association between several functional polymorphisms in the core promoter region of the $\alpha_7$-neuronal nicotinic receptor subunit gene (CHRNA7) and both schizophrenia and the P50 sensory inhibitory deficit (33). The core promoter region is a 231-base pair fragment that is proximal to the ATG translation start site of the CHRNA7. This region of DNA on chromosome 15q13-q14 contains consensus binding sites for several important transcription factors, including stimulating protein Sp1, activator protein AP-4, and a corticosteroid-responsive element, SRE. Polymorphisms in the core promoter region of the full-length CHRNA7 gene were examined in 195 schizophrenia patients from 166 families and 165 control subjects using single-stranded conformational polymorphism analysis (SSCP) and DNA sequencing. A variety of variants were identified in the core promoter region, many of which lie in consensus binding sequences for transcription factors. The prevalence of these promoter variants was statistically significantly higher among the patients with schizophrenia than the control subjects (33). Moreover, an in vitro analysis of the functional significance of some of these “promoter variants” showed that they resulted in a decreased transcription of a luciferase reporter gene (33). These data suggest that some of the polymorphisms in the core promoter region could lead to decreased expression of the $\alpha_7$-neuronal nicotinic receptor subunit, which has been reported in human postmortem hippocampus, reticular thalamic nucleus, and frontal cortex of schizophrenia patients. In an analysis of a possible association between the P50 auditory sensory gating deficit and promoter variants in a subset of 151 control subjects, a relation was found between promoter variants and decreased sensory processing (33). These data support the P50 auditory gating deficit as an important endophenotype in schizophrenia. Further, abnormally regulated transcription of a functional intact receptor could explain the regionally selective decreases in the density of $\alpha_7$ nicotinic cholinergic receptors observed in autopsied brains obtained from patients with schizophrenia. The presence of functionally intact receptors would encourage $\alpha_7$ nicotinic cholinergic receptor agonist strategies for pharmacotherapeutic intervention.

### $\alpha_7$ Nicotinic Cholinergic Receptor Abnormalities in Schizophrenia: Therapeutic Implications

Recently, galantamine HBr, a modest inhibitor of acetylcholinesterase and a positive allosteric modulator of nAChRs, has been marketed and approved for the treatment of Alzheimer’s disease (34). Because galantamine does not cause total inhibition of acetylcholinesterase activity, in addition to prolonging the lifetime and concentration of acetylcholine within cholinergic synapses, it would not be expected to deplete the neurotransmitter pool of choline, a selective $\alpha_7$ nicotinic receptor agonist (4, 35). Further, because of its allosteric modulatory properties, galantamine preserves the nAChR in a sensitive and responsive state, in addition to increasing the efficiency of transduction of the acetylcholine signal (4). In preclinical experiments, galantamine was shown to attenuate the severity of MK-801-elicited popping behavior in mice (36). MK-801-elicited mouse popping behavior serves as a pharmacological model of NMDA receptor hypofunction; MK-801 is a noncompetitive NMDA receptor antagonist that binds to the same hydrophobic channel domain as phencyclidine (37, 38). These data suggest that a delicate balance may exist between NMDA and nicotinic receptor-mediated neurotransmission; furthermore, at least some of the consequences of NMDA receptor hypofunction may be addressed by nicotinic agonist interventions, including positive allosteric modulation. These preclinical data also support and encourage clinical investigation of galantamine in schizophrenia. In fact, our group has reported a very positive therapeutic effect of adjuvant galantamine administration on negative symptoms in a treatment-refractory patient with chronic schizophrenia (35). Galantamine caused a rapid and persistent reduction of negative symptoms, which was sustained over the two-month period of its adjuvant administration. Further, the severity of negative symptoms worsened and re-
turned to their baseline level after its discontinuation. Galantamine was associated with improved grooming, self-care and socialization.

Conclusions

Reduced expression of the $\alpha_7$ nicotinic receptor is associated with deficits in specific psychophysiological functions (e.g., sensory inhibition of the P50 auditory evoked response) that may serve as endophenotypes for schizophrenia. These endophenotypes are phenotypically expressed in unaffected biological relatives of patients with schizophrenia more commonly than in the general population and may be inherited in an autosomal dominant fashion. Interestingly, the reduced density of $\alpha_7$ nicotinic receptors in schizophrenia does not occur homogeneously throughout the brain nor is their complete absence observed. Recent genetic data show that promoter variants (i.e., polymorphisms in a 231-base pair fragment proximal to the start codon of the $\alpha_7$ nicotinic receptor polypeptide subunit on chromosome 15q13-q14) can cause reduced transcription of a reporter gene. Thus, these promoter variants, which are found more commonly among schizophrenia patients than controls, may be responsible for reduced expression of functional $\alpha_7$ nicotinic receptors in selective areas of brain. Conceivably, the existence of these promoter variants and their identification lend themselves to the development of a genetic screening test of increased risk for schizophrenia; however, significant ethical issues would need to be resolved before the development of a genetic screening test of increased risk for schizophrenia can be considered.

The existence of functional receptor protein in the brains of schizophrenia patients, albeit in reduced amounts, supports the development of $\alpha_7$ nicotinic receptor agonist interventions for therapeutic purposes. The effectiveness of selective nicotinic interventions may be enhanced by positive allosteric modulators (e.g., galantamine), which would improve the efficiency of acetylcholine-gated channel opening and help to preserve the receptor in a sensitized state.

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