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Short communication

Phencyclidine-induced cognitive deficits in mice are improved by subsequent subchronic administration of clozapine, but not haloperidol

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Abstract

This study was undertaken to examine the effects of subsequent administration of antipsychotic drugs (clozapine and haloperidol) on cognitive deficits in mice after repeated administration of phencyclidine (PCP). In the novel object recognition test, repeated administration of PCP (10 mg/kg) significantly decreased exploratory preference in the retention test session but not in the training test session. PCP-induced deficits were significantly improved by subsequent subchronic (2 weeks) administration of clozapine (5 mg/kg), but not haloperidol (0.1 mg/kg). These findings suggest that PCP-induced cognitive deficits using the novel object recognition test may be a potential animal model of atypical antipsychotic activity.

Keywords: Schizophrenia; NMDA receptor; Phencyclidine; Cognition; Clozapine; Haloperidol

1. Introduction

Cognitive deficits in patients with schizophrenia are a core feature of the illness, which predicts vocational and social disabilities for patients (Freedman, 2003; Green et al., 2004). Multiple lines of evidence suggest that a dysfunction in the glutamatergic neurotransmission via the *N*-methyl-D-aspartate (NMDA) receptors might be involved in the pathophysiology of schizophrenia (Javitt and Zukin, 1991; Olney and Farber, 1995; Coyle, 1996; Krystal et al., 1999; Hashimoto et al., 2003, 2004, 2005). The NMDA receptor antagonists such as phencyclidine (PCP) are known to induce schizophrenia-like symptoms including cognitive deficits in healthy subjects (Javitt and Zukin, 1991; Krystal et al., 1999). Therefore, PCP has been used as an animal model of cognitive deficits in schizophrenia (Jentsch and Roth, 1999; Mandillo et al., 2003; Sams-Dodd, 1998). It is also well known that the incidence of extrapyramidal side effects of the atypical antipsychotic drug clozapine is lower than that of the typical antipsychotic drug haloperidol, and that clozapine has more efficacy than haloperidol against cognitive deficits in patients with schizophrenia (Potkin et al., 2001), suggesting that atypical antipsychotic drugs could improve cognitive deficits as compared with typical antipsychotic drugs. In the present study, using the novel object recognition test, we examined the effects of subsequent acute or subchronic treatment with antipsychotic drugs (clozapine and haloperidol) on cognitive deficits in mice after repeated administration of PCP.

2. Methods

2.1. Animals

Male ICR mice (6 weeks old) weighing 25–30 g were purchased from SLC Japan (Hamamatsu, Shizuoka, Japan). Mice were housed in the clear polycarbonate cages (22.5 × 33.8 × 14.0 cm) and in groups of 4 or 5 mice under a controlled 12/12-h light–dark cycle (light from 7:00 AM to 7:00 PM), with room

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temperature at 23±1 °C and humidity at 55±5%. The mice were given free access to water and food pellets for mice. The experimental procedure was approved by the Animal Care and Use Committee of Chiba University Graduate School of Medicine.

2.2. Drug administration

PCP hydrochloride was synthesized in our laboratory. Saline (10 ml/kg) or PCP (10 mg/kg expressed as a hydrochloride salt) were administered subcutaneously (s.c.) for 10 days (once daily on days 1–5, 8–12), and no treatment was on days 6, 7, 13 and 14. In a single and acute experiment, 3 days (days 15) after a final administration of saline or PCP, vehicle (10 ml/kg; 0.8% acetic acid), clozapine (5 mg/kg; Novartis Pharmaceuticals, Ltd., Basel, Switzerland)) or haloperidol (0.1 mg/kg; Wako Pure Chemicals Ltd., Tokyo, Japan) were administered intraperitoneally (i.p.). In a separate experiment, 3 days (days 15) after a final administration of saline or PCP, vehicle (10 ml/kg; 0.8% acetic acid), clozapine (5 mg/kg) or haloperidol (0.1 mg/kg) were administered intraperitoneally (i.p.) for consecutive 2 weeks (once daily on days 15–28). The dose of clozapine and haloperidol was selected since these dose were effective in the latent inhibition models of adult offspring of poly I:C treated dams (Zuckerman et al., 2003).

2.3. Novel object recognition test

In the experiment of acute treatment, 1 h after a final administration of vehicle, clozapine or haloperidol, novel object recognition test was performed as previously reported (Tang et al., 1999, 2001). In the experiment of subchronic treatment, novel object recognition test was performed 1 day after a final administration of vehicle, clozapine or haloperidol. The apparatus for this task consisted of a black open field box (50.8 × 50.8 × 25.4 cm). Before the test, mice were habituated in the box for 3 days. During a training session, two objects (various objects differing in their shape and color but similar in size) were placed in the box 35.5 cm apart (symmetrically) and each animal was allowed to explore in the box for 5 min. The animals were considered to be exploring the object when the head of the animal was facing the object within an inch from the object or any part of the body, except for the tail, was touching the object. The time that mice spent exploring each object was recorded. After training, mice were immediately returned to their homecages, and the box and objects were cleaned with 75% ethanol to avoid any possible instinctive odorant cues. Retention tests were carried out at 1-day intervals following the respective training. During the retention test, each mouse was placed back into the same box, in which one of the objects used during training was replaced by a novel one. The mice were then allowed to freely explore for 5 min and the time spent exploring each object was recorded. Throughout the experiments, the objects were used in a counter-balanced manner in terms of their physical complexity. A preference index, a ratio of the amount of time spent exploring any one of the two objects (training session) or the novel one (retention session) over the total time spent exploring respective to both objects, was used to measure memory performance.

2.4. Statistical analysis

Data are expressed as mean±S.E.M. Statistical analysis was performed by using Student *t*-test or one-way analysis of variance (ANOVA) and post hoc Bonferroni test. *P* values less than 0.05 were considered statistically significant.

3. Results

In the novel object recognition test, repeated administration of PCP (10 mg/kg/day for 10 days) caused significant cognitive deficits 3 days (days 15) and 6 weeks (days 57) after a final administration of PCP. In the training session, there was no

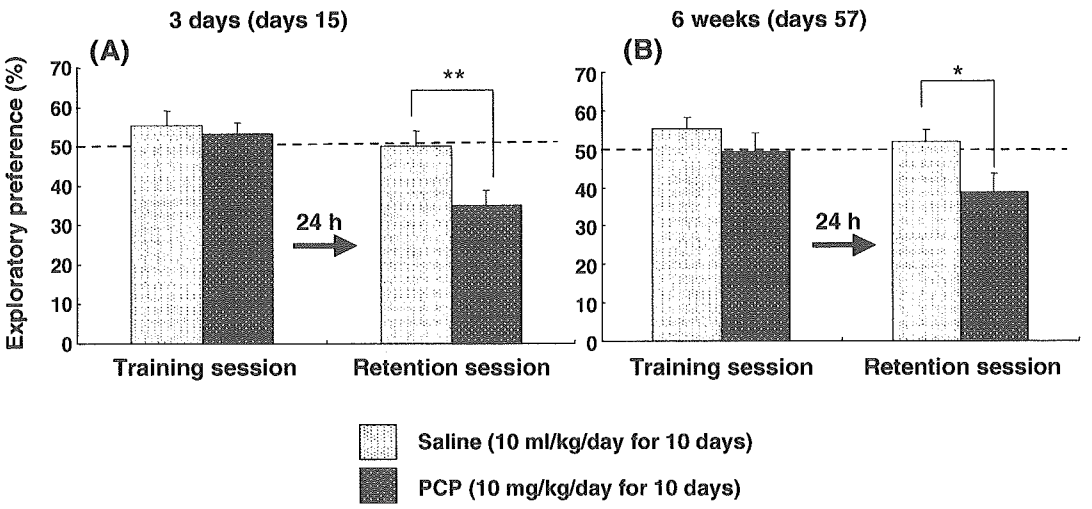

Fig. 1. Repeated administration of PCP caused cognitive deficits in mice. Saline (10 ml/kg, s.c.) or PCP (10 mg/kg, s.c.) were administered for 10 days (once daily on days 1–5, 8–12). In the short-term experiment (3 days after the last administration), the novel object recognition test was performed on days 15 and 16. In the long-term experiment (6 weeks after the last administration), the novel object recognition test was performed on days 57 and 58. The exploratory preference (%) on the *Y* axis is referring to the preference toward the novel object, thus meaning the ability to discriminate between novel object and no-novel object. Values are the mean±S.E.M ($n=7$). $^{*}P<0.05$, $^{**}P<0.01$ as compared with saline-treated group.

difference in the exploratory preference of mice in the PCP-treated and saline-treated control groups (Fig. 1). In the retention session, the exploratory preference of PCP-treated mice was significantly lower than that of mice in the saline-treated group at 3 days ($t=3.14$, $P=0.009$) and 6 weeks ($t=2.20$, $P=0.048$) after a final administration of PCP (Fig. 1). During the training session, there were no significant differences between the two groups in the total amount of time spent exploring two objects.

In the retention session, a single administration of clozapine (5 mg/kg, 1 h) or haloperidol (0.1 mg/kg, 1 h) did not alter reduction of the exploratory preference in mice after repeated administration of PCP (Fig. 2A). In contrast, PCP-induced deficits were significantly improved after subsequent subchronic (2 weeks) administration of clozapine (5 mg/kg/day), but not haloperidol (0.1 mg/kg/day). In the training session, the exploratory preference of four groups was not different (F (3,44)= 1.52, $P=0.224$) (Fig. 2B). However, in the retention session, ANOVA analysis revealed that the exploratory preference of mice in the four groups was significantly different (F (3,44)= 9.44, $P<0.001$) (Fig. 2B). A post hoc Bonferroni test indicated that the exploratory preference of the PCP-treated group was significantly increased after subchronic administration of clozapine ($P<0.001$), but not haloperidol ($P=1.00$) (Fig. 2B).

4. Discussion

The major findings of the present study are that repeated administration of PCP (10 mg/kg/day for 10 days) caused cognitive deficits in mice for a long time (more than 6 weeks after a final administration of PCP), and that PCP-induced cognitive deficits could be improved by subsequent subchronic administration of clozapine, but not haloperidol. In the novel object recognition test, no significant differences in total amount of time spent exploring two objects or in exploratory preference were found between two groups during the training session, suggesting that levels of motivation, curiosity, and interest in exploring novel objects were the same in the two groups. Repeated administration of PCP significantly decreased the exploratory preference in the retention session but not in the training session. In the

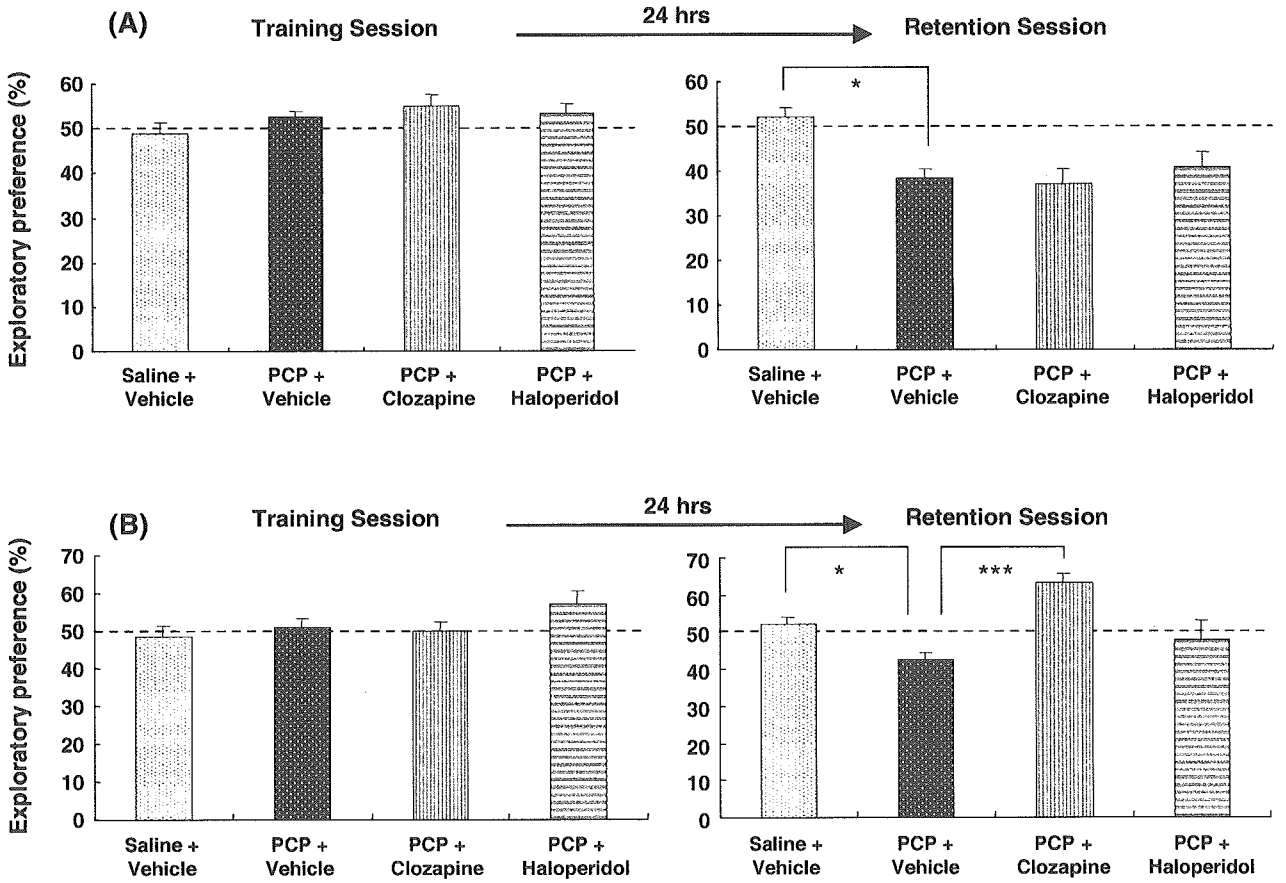

Fig. 2. Effects of clozapine and haloperidol on PCP-induced cognitive deficits in mice. Saline (10 ml/kg) or PCP (10 mg/kg) were administered s.c. for 10 days (once daily on days 1–5, 8–12). (A) Three days (days 15) after the last administration of saline or PCP, vehicle (10 ml/kg; 0.8% acetic acid), clozapine (5 mg/kg) or haloperidol (0.1 mg/kg) were administered i.p. into mice. The novel object recognition test was performed 1 h after administration. Values are the mean±S.E.M ($n=6$–13). *$P<0.05$ as compared with saline-treated group. (B) Three days (days 15) after the last administration of saline or PCP, vehicle (10 ml/kg; 0.8% acetic acid), clozapine (5 mg/kg) or haloperidol (0.1 mg/kg) were administered i.p. into mice for consecutive 2 weeks (once daily on days 15–28). On days 29 and 30, the novel object recognition test was performed. Values are the mean±S.E.M ($n=9$–14). *$P<0.05$ as compared with saline-treated group. ***$P<0.001$ as compared with PCP-treated group.

retention session, the exploratory preference (approximately 40%) of the PCP-treated group was significantly lower than that (approximately 50%) of the saline-treated group, suggesting that the behavior of the PCP-treated mice may not have been due to memory impairment. In contrast, in the 1-h retention session, the exploratory preference (approximately 50%) of the PCP-treated group was significantly lower than that (approximately 60%) of the saline-treated group (data not shown), suggesting that these acute deficits may, in part, be associated with retention memory deficits. Furthermore, it has been reported that repeated administration of PCP caused social interaction deficits in animals (Mandillo et al., 2003; Sams-Dodd, 1998). In addition, negative symptoms such as social withdrawal are related to cognitive deficits in schizophrenic patients (Zakzanis, 1998). Considering these findings, it is likely that our model of PCP-induced cognitive deficits, using the novel object recognition test, may show negative symptoms such as social withdrawal, which are related to cognitive deficits. Interestingly, we found that PCP-induced cognitive deficits could be improved by subsequent subchronic administration of clozapine, but not haloperidol. Therefore, reversal of PCP-induced cognitive deficits, using the novel object recognition test, may be a potential animal model of atypical antipsychotic activity in relation to amelioration of cognitive deficits in schizophrenia.

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α7 Nicotinic Receptor Agonists as Potential Therapeutic Drugs for Schizophrenia

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Abstract: Deficient inhibitory processing of the P50 auditory evoked potential is a measurable marker observed in schizophrenia. Several lines of evidence suggest that α7 nicotinic receptors (α7 nAChRs) play a critical role in P50 auditory sensory gating in the human brain. Similar to schizophrenic patients, DBA/2 mice spontaneously exhibit a deficit in inhibitory processing of the P20-N40 auditory evoked potential, which is a rodent analogue of the human P50 auditory evoked potential. Agonists at α7 nAChRs improve deficient inhibitory processing of the P20-N40 auditory gating potential in DBA/2 mice. In this article, we review the role of α7 nAChRs in the pathophysiology of schizophrenia, and α7 nAChR agonists and indirect agonists (5-hydroxytryptamine-3 (5-HT_3) receptor antagonists, positive allosteric modulators (galantamine, 5-hydroxyindole, PNU-120596), FK960, FR236924) at α7 nAChRs as potential therapeutic drugs for the treatment of schizophrenia. In addition, we also discuss the role of kynurenic acid as an endogenous antagonist of α7 nAChRs in brain.

Keywords: α7 nicotinic receptors, auditory gating, sensory gating, cognition, schizophrenia.

SCHIZOPHRENIA

Schizophrenia is a chronic psychotic mental disorder that affects about one percent of the world's general population. This illness has varied and ominous symptoms that generally begin in late adolescence or early adulthood and usually continue throughout life. Schizophrenia is characterized by three broad types of symptoms: positive symptoms (e.g., hallucinations, delusion, or bizarre behaviors) , negative symptoms (e.g., blunted affect, anhedonia, avolition or apathy, and alogia) , and cognitive impairment (e.g., problems in attention and concentration, psychomotor speed, learning and memory, and executive function) [1,2]. Positive and negative symptoms vary in intensity over time; patients may have predominantly one type at any particular time. In contrast, cognitive impairment is a core feature of the illness, and predicts vocational and social disabilities for patients [1,2].

The cause of schizophrenia is unknown, but several lines of evidence suggest that genetic factors and environmental factors (e.g., prenatal and perinatal events - including maternal influenza, rubella, malnutrition, diabetes mellitus, and smoking during pregnancy - and obstetric complications) contribute to the pathophysiology of this illness [1-9]. Furthermore, schizophrenia may be a neurodevelopmental and progressive disorder with multiple biochemical abnormalities involving the dopamine, serotonin, acetylcholine (ACh) , glutamate, and γ-aminobutyric acid (GABA) systems [1,2,10-23].

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SCHIZOPHRENIA AND SMOKING

In several countries, the prevalence of cigarette smoking among schizophrenic patients is extraordinarily high (70% or greater) [24-29]. While the prevalence of smoking is declining in the general population, it is not declining in the patients with psychiatric disorders, particularly in schizophrenia. It has also been reported that the percentage of schizophrenic patients who quit smoking is much lower when compared with the normal US population [25,30]. The reason underlying the high frequency of smoking in schizophrenic patients is currently unclear. It has also been suggested that smoking may be a form of self-treatment for patients with schizophrenia, since nicotine has been shown to reduce negative symptoms [24-26,28]. Interestingly, it has also been reported that schizophrenic patients are usually smoking before diagnosis [31], suggesting that impaired neurotransmission by nicotine might be involved in the pathophysiology of schizophrenia.

α7 NICOTINIC RECEPTORS

(S)-(-)-Nicotine (Fig. 1, hereafter simply nicotine) , the main addictive component of tobacco, activates and desensitizes nicotinic ACh receptors (nAChRs). In the central nervous system (CNS) , nAChRs normally respond to ACh (Fig. 1) and modulate neuronal excitability and synaptic communication [32-35]. The health consequences of smoking and the mechanisms involved with nicotine dependence continue to be subjects of great interest [36-40]. In addition, recent attention has been directed to the potential role of nAChRs in diseases and therapeutic targets [13,14,41-51].

The nAChRs are ligand-gated ion channels that are distributed all over the human CNS and that each consists of

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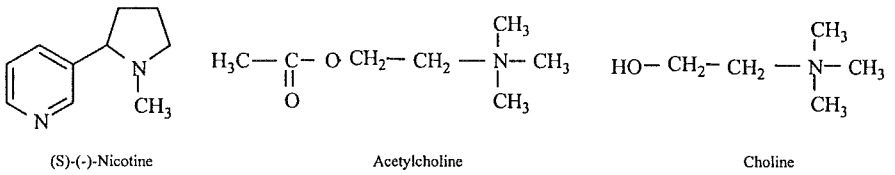


Fig. (1). Chemical structures of (S)-(-)-nicotine, acetylcholine (ACh) and choline.

five subunits (a combination of α and β subunits). At present, nine α (α2-α10) and three β (β2-β4) subunits have been identified and cloned in humans. The numerous combinations of α and β subunits or of α subunits alone can generate many subtypes of nAChRs with different physiologies, pharmacologies and anatomical distributions [35]. Two major subtypes exist in the brain, those comprised of α4β2 and those comprised of α7 subunits (Fig. 2). The former contribute >90% of the high affinity binding sites for nicotine in the rat brain [52]. The low affinity binding sites (α7 subunits) for nicotine are recognized by their nanomolar affinity for α-bungarotoxin [53]. The α7 nAChRs have an unusually high permeability to Ca^{2+} compared to other subtypes and exhibit exceptionally rapid desensitization following exposure to agonists [39,54-58]. Choline (Fig. 1) is an essential physiological component of the cerebrospinal fluid (CSF) and is important for the structural integrity of cell membranes, ACh synthesis, and lipid and cholesterol transport and metabolism. Several lines of evidence suggest that choline is a full agonist of α7 nAChRs, but not other nAChR subtypes [59-62].

The α7 nAChRs are assumed to comprise five α7 subunits and differ from other subtypes of nAChRs. In particular, the α7 nAChRs may play a distinct role in regulating neuronal plasticity. By elevating intracellular Ca^{2+} levels in discrete neuronal locations, these ligand-gated ion channels may influence numerous physiological processes in the developing and adult CNS [35,54,55,63]. Several lines of evidence suggest that both pre- and postsynaptic α7 nAChRs modulate transmitter release in the brain through Ca^{2+}-dependent mechanisms, and that the α7 nAChRs play a role in regulating neuronal growth and differentiation in the developing CNS [35,54,63,64]. Furthermore, it has been proposed that intracellular Ca^{2+} may be coregulated by N-methyl-D-aspartate (NMDA) receptors and α7 nAChRs in the brain [54]. Together with NMDA receptors, postsynaptic α7 nAChRs may serve to regulate intracellular Ca^{2+} levels in neurons, whereas presynaptic α7 nAChRs could serve as a feedback mechanism for modulating glutamatergic transmission (Fig. 3). Thus, it is possible that a close interaction between cholinergic and glutamatergic pathways, mediated by α7 nAChRs and NMDA receptors, may play a role in the pathophysiology of schizophrenia.

SCHIZOPHRENIA AND α7 nAChRs

The binding sites (α7 subtype) labeled by [^{125}I]α-bungarotoxin are different from the high affinity binding sites (α4β2 subtype) for nicotine [65]. In the hippocampus, [^{125}I]α-bungarotoxin binds most intensely to inhibitory interneurons in the CA3 region of Ammon's horn and in the hilus of the dentate gyrus [66]. These authors subsequently suggested that inhibitory interneurons with α7 nAChRs are possible candidates for medication of the habituation of auditory responses in the hippocampus because activation of the interneurons *via* α7 nAChRs would increase the inhibitory synaptic input to pyramidal neurons and thereby diminish the responsiveness of these pyramidal neurons to sensory stimulation [67]. This parallels studies of postmortem human

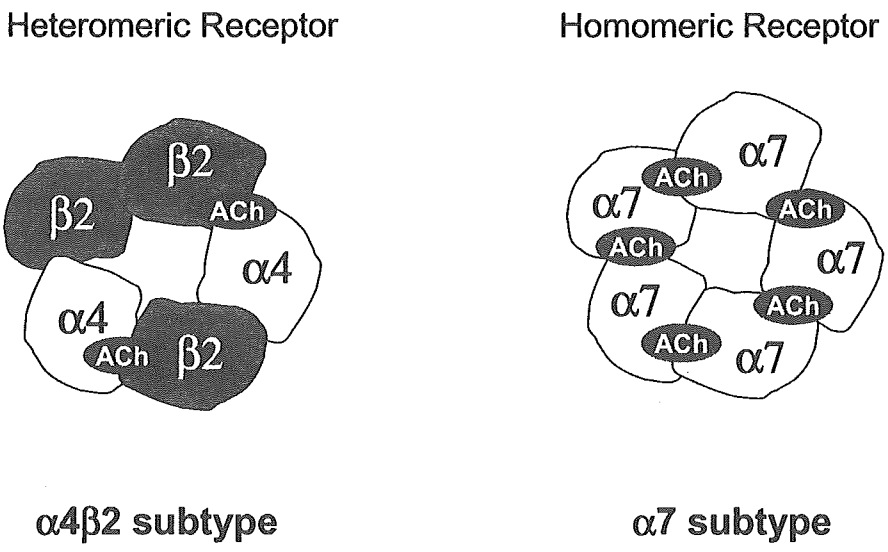


Fig. (2). Receptor assembly of α4β2 subunits and α7 subunits of nicotinic receptors in brain.

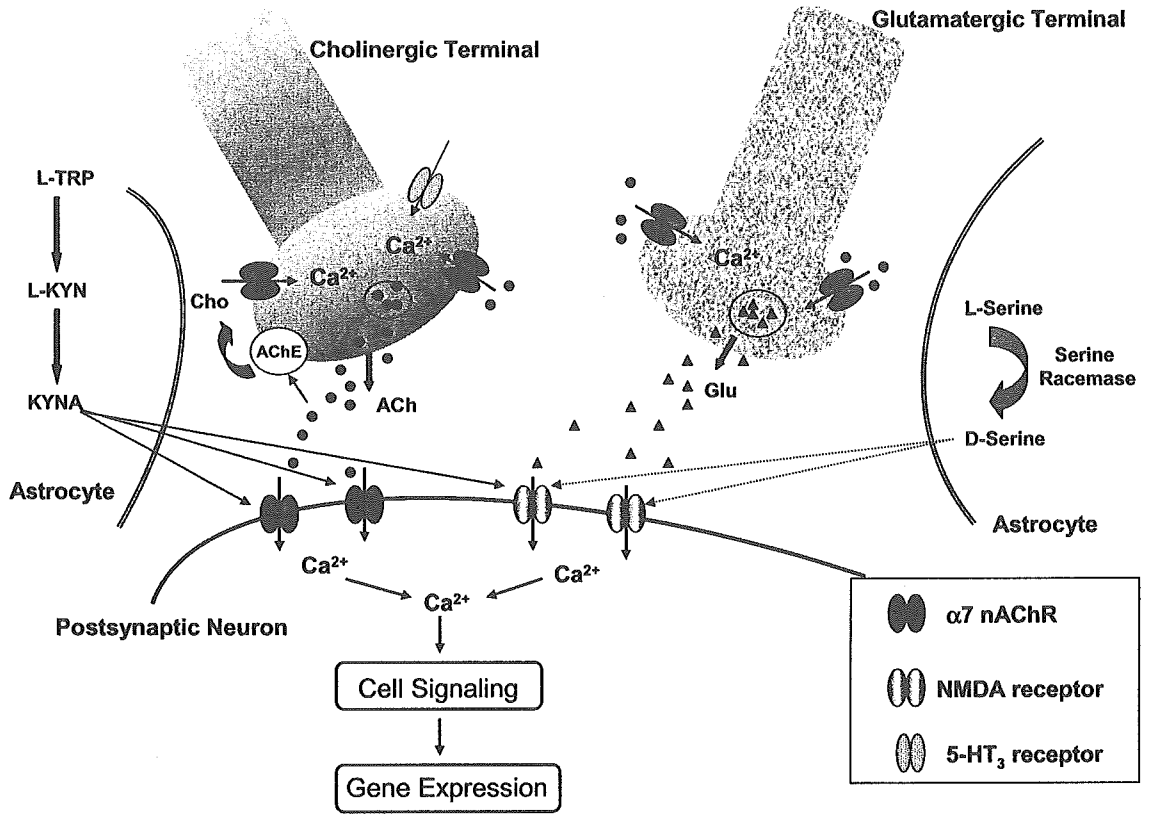


Fig. (3). Role of α7 nicotinic receptors (α7 nAChRs) and NMDA receptors in the neuron-glia communication in the brain. Acetylcholine (ACh) released from the nerve terminal of cholinergic neurons binds to α7 nAChRs on the postsynaptic neurons. By stimulation at α7 nAChRs on the presynaptic terminals, glutamate released from the nerve terminals of glutamate neurons binds to NMDA receptors on the postsynaptic neurons. Thus, the increase in intracellular Ca^{2+} that arises from activation of α7 nAChRs and NMDA receptors lead to cell signaling and gene expression. Kynurenic acid synthesized from L-tryptophan in astrocytes, and, as a non-competitive antagonist, kynurenic acid binds to α7 nAChRs and glycine sites on NMDA receptors. D-Serine is synthesized from L-serine via serine racemase in astrocytes. Glutamate binds to AMPA receptors on astrocyte that stimulate the release of D-serine. Released D-serine binds to glycine site on NMDA receptors. Thus, α7 nAChRs and NMDA receptors can exert a wide range of influences through Ca^{2+} signals, from changes in synaptic plasticity in the brain.

tissue that documented a decreased expression of hippocampal α7 nAChRs in schizophrenic patients [68]. Thus, it seems that schizophrenic patients have fewer α7 nAChRs in the hippocampus, a condition which may lead to failure of cholinergic activation of inhibitory interneurons, manifest clinically as decreased gating of the response to sensory stimulation [67,69,70].

It has been shown that [^{125}I]α-bungarotoxin binding is reduced in the thalamic reticular nucleus of schizophrenic subjects [71], and that α7 nAChR protein levels are reduced in the frontal cortex in patients with schizophrenia [72]. Furthermore, Marutle *et al.* [73] observed a reduction of α7 nAChRs but an increase of [^{3}H]cytisine binding to α4β2 nAChRs in the cingulate cortex of schizophrenic patients.

In regard to the search for peripheral biological markers for schizophrenia, Perl *et al.* [74] investigated the mRNA levels of α7 nAChRs in peripheral blood lymphocytes of schizophrenic patients and healthy controls. They found a significant reduction of α7 nAChR mRNA levels on lymphocytes of schizophrenic patients [74]. This reduction was not a result of medication, because the non-medicated patients displayed the same levels of reduction in α7 nAChR mRNA. In addition, the possibility that the observed decrease in α7 nAChR mRNA levels resulted from nicotine consumption in smoking was excluded, because healthy smokers exhibited the same levels of α7 nAChR mRNA as non-smokers [74]. These findings suggest that mRNA levels of α7 nAChRs in peripheral blood lymphocytes may serve a reliable peripheral biological marker for schizophrenia.

SENSORY GATING DEFICITS IN SCHIZOPHRENIA AND α7 nAChRs

Deficits of sensory gating in schizophrenia derive from the clinical observation that patients report failures of information processing characterized by poor sensory gating [67,75-77]. The underlying problem is evident in the inability of people with schizophrenia to adequately filter their response to incoming sensory stimulation, as measured by their inhibitory processing of the P50 auditory evoked potential. The P50 auditory evoked potential is a positive electroen-

cephalographic waveform that occurs 50 msec after presentation of an auditory stimulus. When pairs of auditory stimuli are presented, with a 500 msec interstimulus interval, schizophrenic patients fail to adequately inhibit the P50 response to the second stimulus. Normal subjects, however, have significantly reduced responses to the second stimulus [67,75,78,77]. The reduced response to the second stimulus reflects inhibitory processing of the information that may function to protect the individual from being overwhelmed by incoming, repetitive sensory information. It is known that nicotine transiently normalizes the P50 auditory evoked-potential deficits in schizophrenic patients [79,80].

Genetic linkage analysis of the P50 auditory-evoked potential deficit in families of patients with schizophrenia has revealed a peak LOD score at 15q13-q14, and the LOD score was 5.3 (theta=0.00) at the D15S1360 marker, which is located in intron 2 of the gene for the α7 nAChR subunit (CHRNA7) [81]. The CHRNA7 gene is located on chromosome 15q13-q14, a region linked with schizophrenia in several earlier studies [82,83]. The CHRNA7 has a partial duplication of exons 5-10, including the intervening introns (CHRFAM7) that map approximately 0.5 Mb proximal to the full-length CHRNA7 gene [84]. The D15S1360 microsatellite repeats, in intron 2 of the CHRNA7 gene, cosegregate with an auditory gating deficit in family linkage studies of schizophrenic patients [81]. Furthermore, in a mutation screening of the CHRNA7 gene from schizophrenics and controls, Leonard *et al.* [85] identified the promoter polymorphisms that decreased the subunit transcription and P50 inhibition in schizophrenia. Moreover, an association has been demonstrated between the homozygous 113 bp allele on D15S1360 polymorphism of the CNRNA7 gene and smoking risk in schizophrenia [86]. Taken together, these results suggest that the CHRNA7 gene is likely susceptible to the deficits of sensory gating P50 in schizophrenia [12,13,29,67,69,70,76,81,85].

α7 NICOTINIC RECEPTOR AGONISTS

While there are a number of nicotinic receptor agonists known to be selective for the α4β2 subtype, there are some agonists which bind the α7 nAChRs selectively over other subtypes [42,47,49]. Anabaseine (2-(3-pyridyl)-3,4,5,6-tetrahydropyridine) (Fig. 4) , a naturally occurring substance in nemertines, is an agonist at the neuromuscular junction [87] and is structurally related to nicotine. The more well known compound anabasine (neonicotine; 3-(2-piperidinyl) pyridine) (Fig. 4) is a weak nicotinic alkaloid found in tobacco that lacks the imine double bond present in anabaseine. Three analogues of anabaseine, 3-(2,4)-dim-ethoxy-benzilidine anabaseine (DMXB-A; also known as GTS-21), 3-(4)-dimethylaminobenzylidine anabaseine (DMAB), and 3-(4)-dimethylaminocinnamylidine (DMAC) , have been reported to be functionally selective for the α7 nAChRs [88] (Fig. 4). Compared with anabaseine and the other derivatives, DMAC was the most potent at displacing [^{125}I]α-bungarotoxin binding (putative α7 subtype) and the least potent at displacing [^{3}H]cytisine binding (putative α4β2 subtype) to brain membranes. These anabaseine derivatives were partial agonists at α7 nAChRs [88]. Furthermore, DMXB-A bound to human α4β2 nAChRs (Ki=20 nmol/L) 100-fold more potently than to human α7 nAChRs, and was 18- and 2-fold less potent than (-)-nicotine at human α4β2

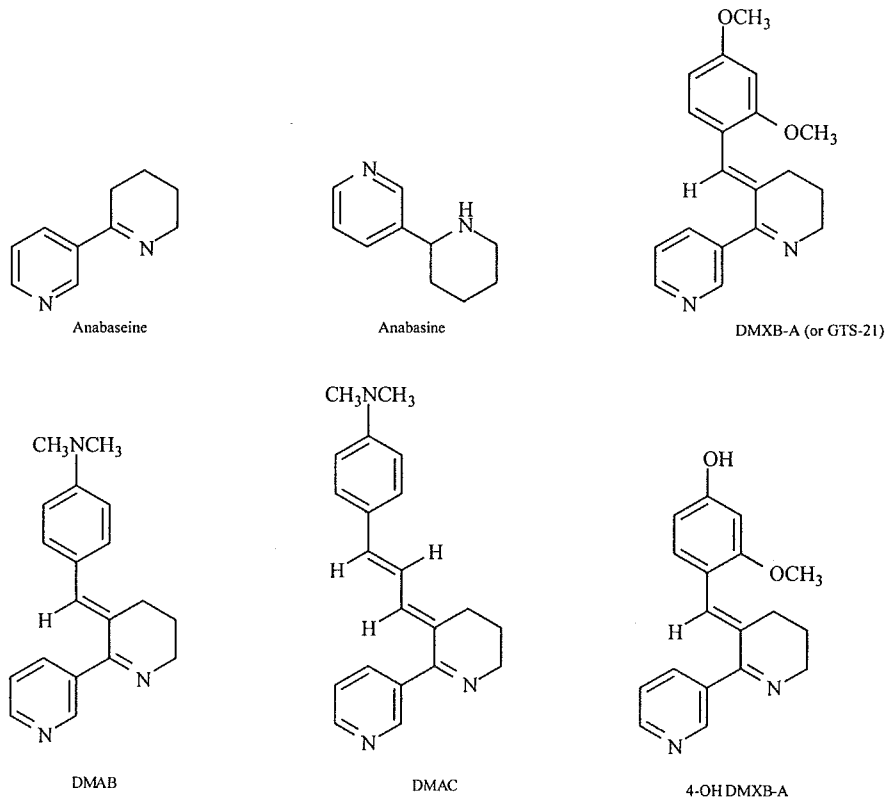


Fig. (4). Chemical structures of anabaseine, anabasine, DMXB-A, DMAB, DMAC and OH-DMXB-A.

and α7 nAChRs, respectively [89]. The primary human metabolite, 3-(4-hydroxy-2-methoxybenzylidine) anabaseine (4-OH DMXB-A; Fig. 4) , of DMXB-A exhibited a similar level of efficacy for human α7 nAChRs [90,91]. Initial (phase I) clinical studies on DMXB-A have been reported [92]. DMXB-A was administered to 87 healthy volunteers. Initially, the effects of single doses (range, 1-250 mg) were assessed. The elimination half-life ranged between 0.5 and 1.0 h for DMXB-A and its major phase I metabolite, 4-OH DMXB-A. No serious adverse effects were reported with these doses. At twice daily doses of 75 and 150 mg for 5 days, DMXB-A improved the cognitive function of young adult volunteers. Furthermore, DMXB-A improved long-term memory as well as working memory and attention, as measured by the Cognitive Drug Research test battery [92]. Therefore, it is of great interest to examine the effects of DMXB-A in schizophrenic patients, who have been shown suggested to have low levels of deficits in the functioning of α7 nAChRs [68].

Researchers at Astra Zeneca reported the profile of AR-R17779, (-)-spiro[1-azabicyclo[2.2.2]octane-3,5'-oxazolidin-2'-one] (Fig. 5) , a potent full agonist of the α7 nAChRs that is highly selective for the α7 subtype over the α4β2 subtypes [93]. AR-R17779 was prepared as shown in Fig. 5. Reaction of methyl 3-hydroxy-1-azabicyclo-[2.2.2]octane-3-acetate with hydrazine followed by Curtius rearrangement led to the desired carbamate. Resolution of the carbamate was accomplished via the dibenzoyl-D (or L)-tartrate salt. AR-R17779 has been widely used as a selective full agonist at the α7 nAChRs. For example, AR-R17779 failed to stimulate locomotor activity in both nicotine-nontolerant and -sensitized rats, whereas nicotine and the putative agonist SIB1765F, [±]-5-ethynyl-3-(1-methyl-2-pyrrolidinyl) pyridine fumarate (Fig. 6) [92], at α4β2 nAChRs increased the activity under both experimental conditions, suggesting a negligible role of α7 nAChRs in nicotine-induced hyperlocomotion and reward in the rat [95]. Furthermore, it has been reported that chronic administration of both nicotine and SIB1765F, but not AR-R17779, resulted in an enhanced locomotor response to acute challenge with either nicotine or SIB1765F but not AR-R17779, suggesting that the α4β2 subtype plays a role in both the initiation and expression of sensitization to the psychomotor stimulant effects of nicotine [96]. Moreover, administration of AR-R17779 improved learning in two radial-arm maze tasks and reversed working memory impairment caused by fimbria-fornix section [97]. These findings suggest that α7 nAChRs play a role in learning and memory, and that agonists at α7 nAChRs might have therapeutic potential for cognitive impairments in neuropsychiatric diseases, including schizophrenia.

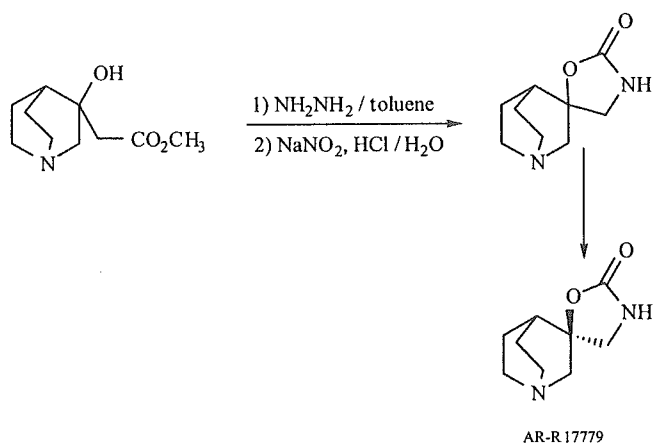
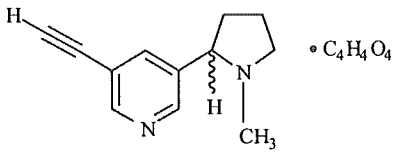


Fig. (5). Synthesis of AR-R17779.



SIB 1765F

Fig. (6). Chemical structure of SIB1765F.

Macor and Wu reported some derivatives of 1-azabicyclo[2.2.2]oct-3-yl phenylcarbamate as agonists at α7 nAChRs [98] (Fig. 7). In order to develop novel agonists at α7 nAChRs, we hypothesized that 1-azabicyclo[2.2.2]octane

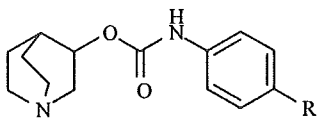


Fig. (7). 1-Azabicyclo[2.2.2]oct-3-yl phenylcarbamate derivatives.

derivatives, bearing an aromatic part and a spacer group at the 3-position, may have agonist activity at α7 nAChRs [99] (Fig. 8). We reported a synthetic approach and the structure activity relationship of 3-substituted 1-azabicyclo [2.2.2]

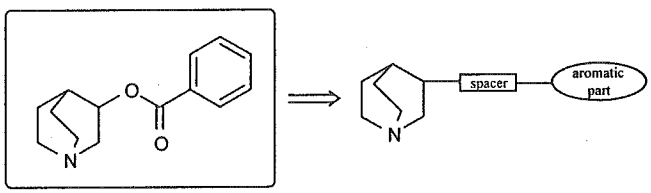


Fig. (8). Synthetic strategy for α7 nAChR agonists.

octane derivatives. As shown in Table 1, the introduction of an α or β-naphthyl moiety as the aromatic part dramatically enhanced the affinity at α7 nAChRs (Table 1). Moreover, the replacement of the naphthyl part with the benzo[b]thiophen-2-yl increased the affinity for α7 nAChRs (Table 1). The (S)-stereoisomer of the benzo[b]thiophen derivative was more potent than that of the (R)-isomer; however, the compound showed poor bioavailability [99]. Table 2 shows the binding data of benzo[b]thiophen-2-yl derivatives. Alkylene analogues were more potent than their corresponding ketone analogues [99]. Among these derivatives, (+)-3-[2-(benzo[b] thiophen-2-yl)-2-oxoethyl]-1- azabicycle[2.2.2] octane is a potent and partial agonist of α7 nAChRs [99] (Fig. 9). Furthermore, we reported the structure-activity relationships and pharmacokinetic profiles of the series of compounds leading

Table 1. Binding Affinities of 1-Azabicyclo[2,2,2]octane Derivatives

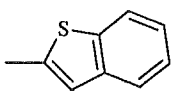
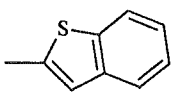
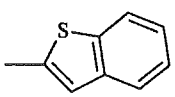
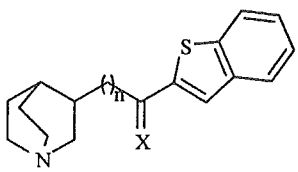
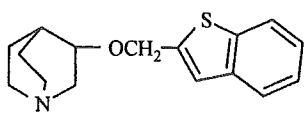
Spacer	Aromatic part	α7 affinity (IC_{50}; μmol/L)
— OCO—	Ph	2.6
— OCH_2—	Ph	3.7
— OCH_2—	2-Naphthyl	0.15
— OCH_2—	1-Naphthyl	0.73
— OCH_2—		0.059
— OCH_2—	 (R)	0.515
— OCH_2—	 (S)	0.026

Table 2. Binding Affinities of Benzo[b]thiophen-2-yl Derivatives



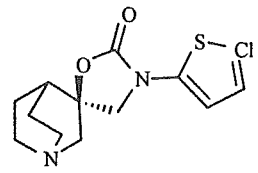
N	X	α7 affinity (IC_{50}; μmol/L
0	O	0.95
0	H_2	0.11
1	O	0.15
1	H_2	0.023
2	O	1.7
2	H_2	0.37



(+)-3-[2(benzo[b]thiophen-2-yl)-2-oxoethyl]-1-azabicycle[2.2.2]octane

Fig. (9). Chemical structure of (+)-3-[2-(benzo[b]thiophen-2-yl)-2-oxoethyl]-1- azabicycle[2.2.2]octane.

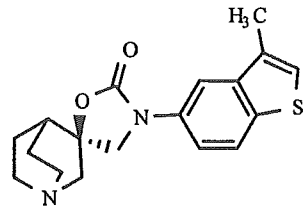
to the discovery of (R)-3' (5-chlorothiophen-2-yl) spiro-1-azabicyclo[2,2,2]octane-3,5'-[1',3']oxazolidin-2'-one [100] (Fig. **10**) This compound has potent binding affinity (Ki = 9 nmol/L for α7 nAChRs) and good selectivity toward the other nicotinic subtypes (α4β2 and α1β2γδ). Also, this compound has good oral bioavailability and brain permeability.



(R)-3'(5-chlorothiophen-2-yl)spiro-1-azabicyclo[2,2,2]octane-3,5'-[1',3']oxazolidin-2'-one

Fig. (10). Chemical structure of (R)-3'(5-chlorothiophen-2-yl)spiro-1-azabicyclo[2,2,2]octane- 3,5'-[1',3']oxazolidin-2'-one.

Interestingly, this compound (10 mg/kg, p.o.) significantly improved dizocilpine ((+)-MK-801) (3 mg/kg)-induced auditory gating deficits in rats, suggesting that this compound has the potential to improve sensory gating deficits in schizophrenic patients [100]. Moreover, we have developed a novel partial α7 nAChR agonist, W-56203, (R)-3'-(3-methylbenzo [b]thiophen-5-yl) spiro[1- azabicyclo[2.2.2] octane-3,5'-oxazolidin]-2'-one, (Fig. **11**) at α7 nAChRs (Katayama *et al.,* The 33rd SFN Annual Meeting, 2003). W-56203 bound to α7 nAChRs with a Ki value (3 nmol/L). No significant binding of W-56203 was detected at α4β2



W-56203

Fig. (11). Chemical structure of W-56203.

nAChRs or muscarinic receptors. Furthermore, W-56203 showed no binding to other known receptors (dopamine D_1 and D_2, 5-HT_{1A}, 5-HT_2, adrenergic α1, α2, histamine H_1 and H_2) or ion channels (NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)) , although W-56203 exhibited a moderate affinity to 5-HT_3 receptors. In cultured hippocampal neurons, W-56203 evoked a rapidly desensitizing inward current, which was blocked by the selective α7 nAChR antagonist methyllycaconitine (MLA, 1 nmol/L) (Fig. **12**). Interestingly, W-56203 has been shown to significantly improve dizocilpine-induced auditory gating deficits in rats (Katayama *et al.,* The 33rd SFN Annual Meeting, 2003). These results suggest that W-56203 is an orally active and partial agonist at α7 nAChRs, and that W-56203 would be a useful drug for the treatment of schizophrenia.

Researchers at Targacept Inc. reported that 2-(3-pyridyl)-1-azabicyclo[3.2.2]nonane (TC-1698; Fig. **13**) was a highly selective agonist of α7 nAChRs [101]. TC-1698 exhibited a

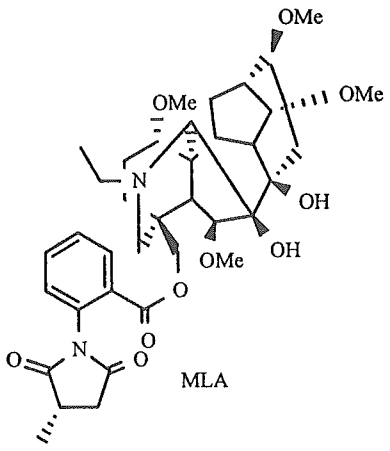


Fig. (12). Chemical structure of MLA.

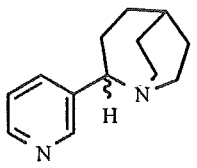


Fig. (13). Chemical structure of TC-1698.

Ki of 11 nmol/L in the binding assay of [^{3}H]MLA to rat hippocampal membranes, whereas TC-1698 (10 μmol/L) had no or very low affinity for other receptors. TC-1698 exerts neuroprotective effects via activation of the JAK2/PI3K cascade, which can be neutralized through activation of the angiotensin II receptors [101]. These findings suggest that JAK2 plays a central role in the α7 nAChR activation of the JAK2-PI3K cascade in PC12 cells, which ultimately contribute to α7 nAChR mediated neuroprotection.

Researchers at Pfizer reported the selective α7 nAChR agonist PNU-282987, N-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]-4-chlorobenzamide hydrochloride (Fig. **14**) [102, 103]. PNU-282987 binds to α7 nAChR with a Ki of 27 nmol/L, and showed evoked whole-cell currents from cultured rat hippocampal neurons that were sensitive to the selective α7 nAChR antagonist MLA [102,103]. Systemic administration of PNU-282987 (1 mg/kg, i.v.) significantly improved d-amphetamine-induced sensory gating deficits in chloral hydrate-anesthetized rats. These findings suggest that PNU-282987 may be useful for treating the cognitive and attentional deficits of schizophrenia [102,103].

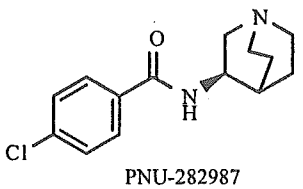


Fig. (14). Chemical structure of PNU-282987.

Very recently, researchers at Sanofi-Aventis demonstrated a novel α7 nAChR agonist SSR180711A, 4-bromophenyl 1,4-diazabicyclo[3.2.2]nonane-4-carboxylate hydrochloride (Fig. **15**; Biton *et al.*, The 34th SFN Annual Meeting, 2004). Binding assays show that SSR180711A has a high and selective affinity for the human (Ki=78 nmol/L) and rat (Ki=50 nmol/L) α7 nAChRs. This ligand inhibits, in a dose-dependent manner, the *ex vivo* [^{3}H]α-bungarotoxin binding in mouse cortical homogenates after both i.p. and p.o. administration. At recombinant human α7 nAChRs, SSR180711A displays a partial agonist profile. Furthermore, it has been reported that SSR180711A improves cognitive deficits in a variety of rat models related to schizophrenia. This drug restores the selective attention deficit induced by PCP administration at the neonatal stage. This action is reversed by the selective α7 nAChR antagonist MLA, suggesting that α7 nAChRs play a role in the mechanism of action of SSR180711A (Pichat *et al.*, The 34th SFN Annual Meeting, 2004). This drug also restores a short-term episodic memory impairment and a spatial working memory deficit induced by PCP or dizocilpine. In addition, it has been shown to enhance the *in vivo* retrosplenial cortex firing in rats (Pichat *et al.*, The 34th SFN Annual Meeting, 2004). The retrosplenial cortex is known to be a corticolimbic structure involved in neuropathological changes related to NMDA receptor hypofunction [104-110]. This drug also enhances extracellular dopamine release in the prefrontal cortex (Pichat *et al.*, The 34th SFN Annual Meeting, 2004). Taken together, these findings suggest that SSR180711A has a potential to improve cognitive deficits associated with schizophrenia. Therefore, future clinical studies of SSR180711A on cognitive deficits in schizophrenic patients would be of great interest.

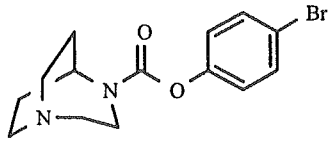


Fig. (15). Chemical structure of SSR180711A.

ANIMAL MODELS OF SENSORY GATING DEFICITS AND α7 NICOTINIC RECEPTOR AGONISTS

The hippocampal P20-N40 wave in DBA/2 mice has been used to model the neurobiology and pharmacology of the human P50 processing deficit [111-115]. Inhibition of the P20-N40 response and expression of α7 nAChRs in the hippocampus were found to be significantly correlated across nine inbred strains of mice [112]. This correlation showed that mouse strains with the fewest hippocampal α7 nAChRs had the least inhibition of the P20-N40 response to the second of paired stimuli [112]. In particular, the DBA/2 strain of inbred mice was shown to fail to attenuate their response to the second stimulus and to have significantly decreased expressions of the α7 nAChRs in their hippocampi [112]. This parallels studies of postmortem human tissue that documented the decreased expression of hippocampal α7 nAChRs in schizophrenic patients [68-70]. Interestingly, subcutaneous or intragastric injection of DMXB-A has been demonstrated to normalize deficient P20-N40 inhibition in

DBA/2 mice [113,114]. Therefore, agonists at α7 nAChRs are a drug candidate that may prove efficacious in normalizing deficient P50 processing in schizophrenic patients [12,13,29].

α7 nAChRs AND 5-HYDROXYTRYPTAMINE-3 (5-HT_3) RECEPTORS

Both α7 nAChRs and 5-hydroxytryptamine-3 (5-HT_3) receptors are members of the superfamily of ligand-gated ion channels [56,116-118] (Fig. **16**). These two receptors share the greatest similarity within the family, displaying approximately 30% sequence homology [119]. Tropisetron and ondansetron (Fig. **17**) are potent 5-HT_3 receptor antagonists that are widely used in the treatment of patients with chemotherapy-induced or postoperative nausea and vomiting [120,121]. It has been reported that tropisetron is a partial agonist of α7 nAChRs with a high affinity, whereas ondansetron is a weak agonist of α7 nAChRs [122,123]. Macor *et al.* [122] synthesized the replacement of the azabicyclo[3.2.1]heptane amine in tropisetron with the azabicyclo[2.2.2]hexamine amine. 1-Azabicyclo[2.2.2]oct-3-yl 1H indole-3-carboxylate and 1-azabicyclo[2.2.2]oct-3-yl 1H indole-1-carboxylate (Fig. **18**) were found to be partial agonists of α7 nAChRs with a high affinity, whereas they also bound to 5-HT_3 receptors with high affinities [122]. We found that tropisetron improves the deficient inhibitory processing of P20-N40 in DBA/2 mice, and that improvement of tropisetron could be antagonized by coadministration of the selective α7 nAChR antagonist MLA [124]. These findings suggest that tropisetron improves abnormal auditory gating of P20-N40 in DBA/2 mice via α7 nAChRs. Recently, Adler *et al.* [125] demonstrated that ondansetron, a highly selective 5-HT_3 receptor antagonist, improves deficits of P50 suppression in medicated schizophrenic patients [125]. In addition, we have found that tropisetron improves deficits of P50 suppression in schizophrenic patients [126]. Taken together, these results indicate that both agonist activity at α7 nAChRs and antagonist activity at 5-HT_3 receptors for tropisetron might be implicated in the therapeutic action of normalization of P50 suppression by tropisetron. Furthermore, it would be of great interest to study the effects of tropisetron on cognitive dysfunction in schizophrenic patients, since P50 auditory gating deficits have been associated with attentional deficits in schizophrenia [77,127].

It has been reported that PSAB-OFP, (R)-(-)-5'-phenylspiro[1-azabicyclo[2.2.2] octane-3,2'-(3'-H) furo[2,3-b]pyridine (Fig. **19**) , binds to human recombinant α7 nAChRs. However, PSAB-OFP also displayed high affinity binding to 5-HT_3 receptors. These results show that PSAB-OFP is an equipotent, partial agonist of both α7 nAChRs and 5-HT_3 receptors [128]. It has been found that agonist activity on one receptor translates to antagonist activity at the other receptor. For example, the endogenous ligands, ACh and 5-HT, act as antagonists on their counterparts [129,130]. The 5-

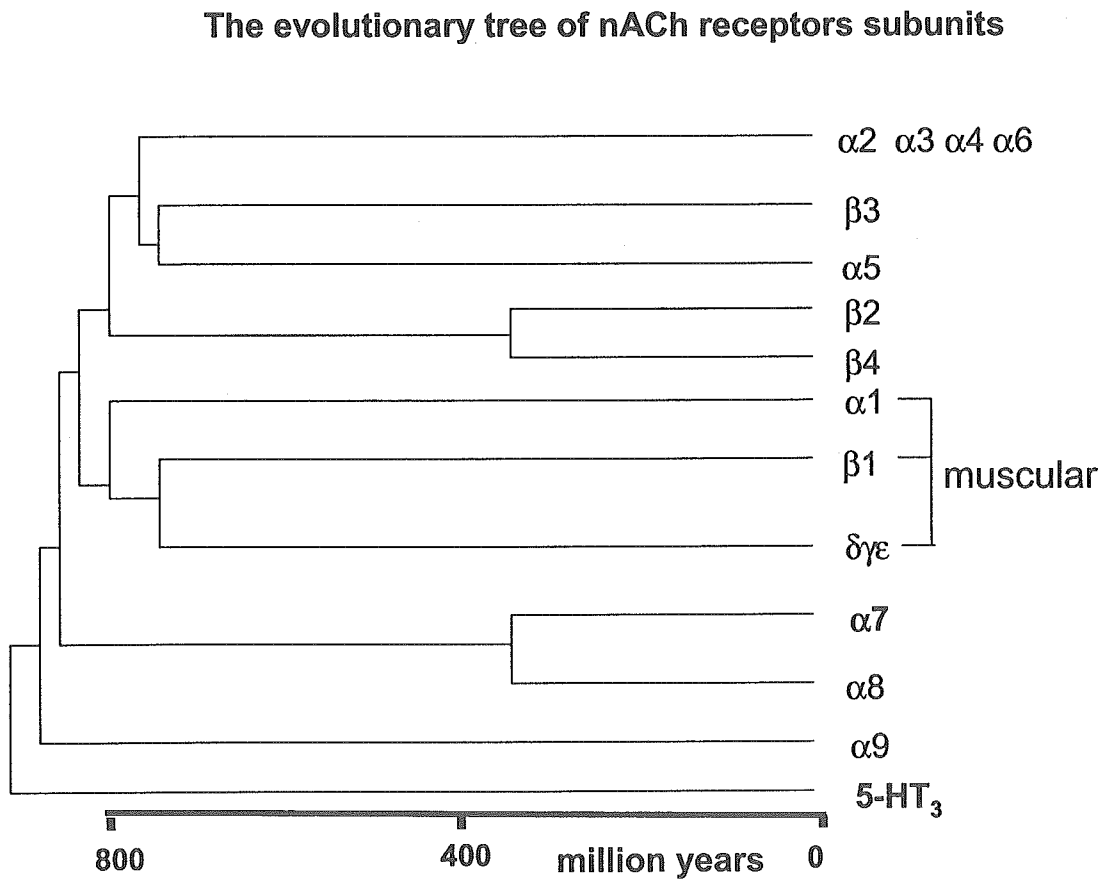
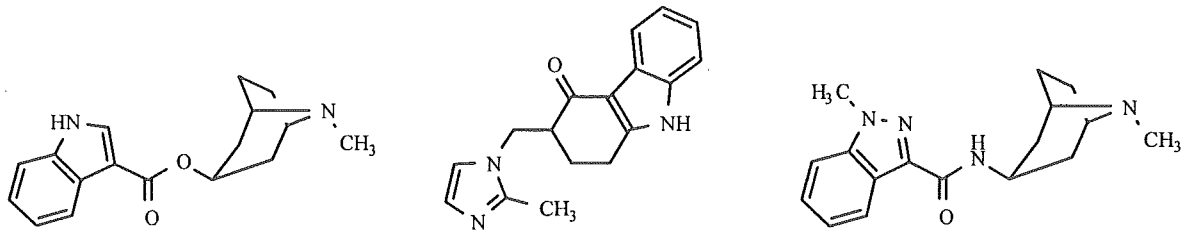


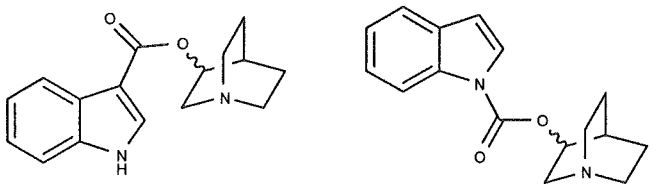
Fig. (16). The evolutionary tree of nACh receptors subunits and 5-HT_3 receptors.



Tropisetron Ondansetron LY-278,584

Fig. (17). Chemical structures of 5-HT_3 receptor antagonists (tropisetron, ondansetron, LY-278,584).

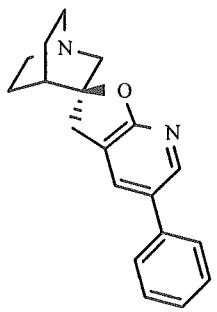
HT_3 receptor antagonist tropisetron, but not ondansetron or LY278,584, is a partial agonist of the α7 nAChRs [122]. The dramatic influence of small changes in ligand [131] or receptor structure [132] on the functional activity at α7 nAChRs and 5-HT_3 receptors might provide an understanding of the basis of the cross-reactivity and thereby allow development of selective ligands.



1-Azabicyclo[2.2.2]oct-3-yl 1H indole-3-carboxylate

1-Azabicyclo[2.2.2]oct-3-yl 1H indole-1-carboxylate

Fig. (18). Chemical structures of 1-azabicyclo[2.2.2]oct-3-yl 1H indole-3-carboxylate and 1-azabicyclo[2.2.2]oct-3-yl 1H indole-1-carboxylate.

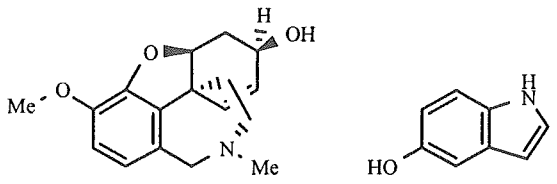


PSAB-OFP

Fig. (19). Chemical structure of PSAB-OFP.

POSITIVE MODULATORS OF α7 nAChRs

Positive allosteric modulators potentiate the response to nicotinic receptor agonists by acting at a site other than the agonist binding site or channel area. Galantamine (acetylcholinesterase inhibitor) (Fig. **20**), an approved drug for treatment of Alzheimer's disease, is also a potent allosteric potentiator of nicotinic receptors, including α7 nAChRs [133-136]. Galantamine has been reported to improve negative symptoms in patients with schizophrenia [137]. Furthermore, the combination of galantamine with risperidone has shown therapeutic benefits for two treatment-resistant patients with schizophrenia whose successful treatment with clozapine had been discontinued because of agranulocytosis [138]. Thus, it is likely that this dual action (cholinesterase inhibition and allosteric potentiation of nAChRs) may be implicated in the mechanisms of action of galantamine.

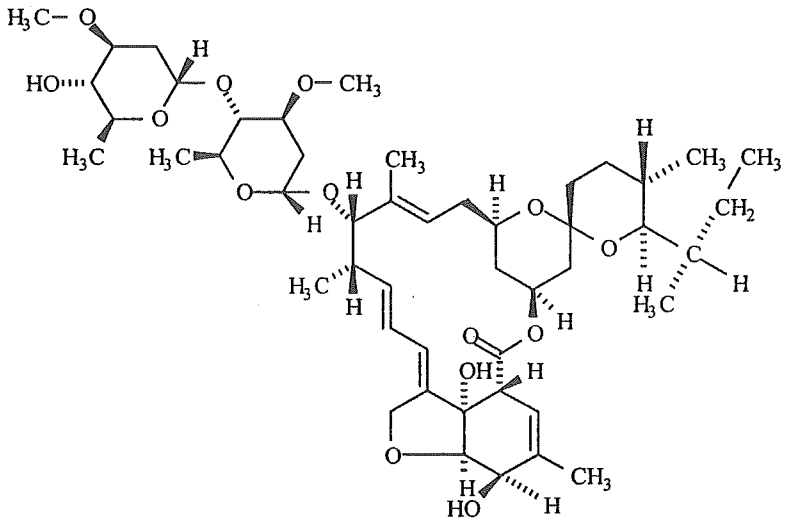


Galantamine 5-Hydroxyindole

Fig. (20). Chemical structures of galantamine and 5-hydroxyindole.

Ivermectin (Fig. **21**), a semisynthetic analogue of the natural compound avermectin, has been considered the drug of choice for the treatment of river blindness (*Onchocerciasis*) [139]. Ivermectin enhanced ACh-evoked current of neuronal human α7 nAChRs, and increased apparent affinity and cooperativity of the dose-response curve, suggesting that ivermectin acts as a positive allosteric effector of α7 nAChRs [140].

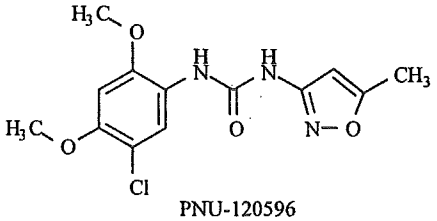
In has been reported that 5-hydroxyindole (Fig. **20**) potentiates 5-HT_3 receptors [141-144]. It is, therefore, of interest to examine the effects of 5-hydroxyindole on α7 nAChRs, because α7 nAChRs belong to the superfamily of ligand-gated ion channels, including 5-HT_3 receptors. Gurley *et al.* (The 30th SFN Annual Meeting, 2000) reported that α7 nAChRs could be potentiated by 5-hydroxyindole. In *Xenopus* oocytes expressing α7 nAChRs, 5-hydroxyindole potentiated sub-maximal ACh (60 μmol/L)-induced ion currents in a concentration-dependent manner, although 5-hydroxyindole itself did not act as an agonist of α7 nAChRs [145]. Furthermore, it has been reported that 5-hydroxyindole potentiated α7 nAChR-mediated increases in intracellular free Ca^{2+} levels in cells expressing α7 nAChRs, and that the ACh-induced release as well as the 5-hydroxyindole-induced enhancement of release were blocked by the α7 nAChR antagonists, MLA or α-bungarotoxin, suggesting a role of α7 nAChRs [145].



Ivermectin (22,23-dihydroxyavermectin B_{1a})

Fig. (21). Chemical structure of ivermectin.

Researchers at Pfizer reported the novel positive allosteric modulator of the α7 nAChRs, PNU-120596, 1-(5-chloro-2,4-dimethoxy-phenyl)-3-(5-methyl-isoxazol-3-yl)-urea (Fig. 22) [146]. PNU-120596 increased agonist-evoked Ca^{2+} flux mediated by an engineered variant of the human α7 nAChRs. This compound increased the frequency of ACh-evoked GABAergic postsynaptic currents measured in pyramidal neurons, and greatly enhanced the ACh-evoked inward currents in hippocampal interneurons. Interestingly, systemic administration of PNU-120596 (0.1 - 3 mg/kg, i.v.) improved auditory gating deficits induced by administration of d-amphetamine (1 mg/kg). These findings suggest that PNU-120596 represents a new class of molecule that enhances α7 nAChR function and thus has the potential to treat psychiatric and neurological disorders [146].



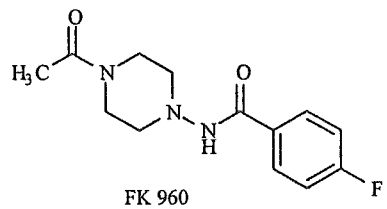
PNU-120596

Fig. (22). Chemical structure of PNU-120596.

Taking these results together, it is likely that the identification of allosteric positive modulators for α7 nAChRs may lead to the design of novel classes of ligands that would be useful therapeutic drugs for neuropsychiatric diseases, including schizophrenia, although the precise mechanisms by which allosteric positive modulators potentiate the α7 nAChR-mediated responses are currently unknown.

FK960 AND FR236924

FK960 (N-(4-acetyl-1-piperazinyl)-p-fluorobenzamide monohydrate) (Fig. 23), which activates somatostatinergic neurotransmission, has considerable potential for the treatment of cognitive impairment, including that associated with Alzheimer's disease [147]. In hippocampal CA1 neurons, FK960 significantly increased the amplitude of the excitatory postsynaptic potentials, and the modulatory action of FK960 was blocked by treatment with MLA or α-bungarotoxin [148]. These findings suggest that FK960 increases the quantal release of glutamate from Schaffer collateral-commissural nerve terminals in the hippocampal CA1 areas either by changing the ambient levels of ACh or by positively modulating the activity of α7 nAChRs on glutamatergic nerve terminals [148]. In considering the role of α7 nAChRs in the pathophysiology of schizophrenia, would be of great interest to study the effects of FK960 in schizophrenic patients, since FK960 has been tried as a therapeutic drug in Alzheimer's disease.



FK 960

Fig. (23). Chemical structure of FK960.

Essential fatty acids, such as arachidonic acid (Fig. 24), linoleic acid (Fig. 24), and linolenic acid (Fig. 24) have been thought to play a role as raw materials for bioactive lipid mediators such as prostaglandins and/or leukotrienes. However, several recent findings suggest that these *cis*-unsaturated free acids induce a long-lasting facilitation of hippocampal synaptic transmission that resembles long-term potentiation, as a result of enhancing the activity of nAChRs via a protein kinase C (PKC) pathway [149-151]. Researchers at Fujisawa Pharmaceutical Co., Ltd. reported a newly synthesized linoleic acid derivative (FR236924; 8-(2-((2-pentylcyclopropan-1-yl) methyl) cyclopropyl) octanoic acid, Fig. 24) with a cyclopropane ring instead of *cis*-double

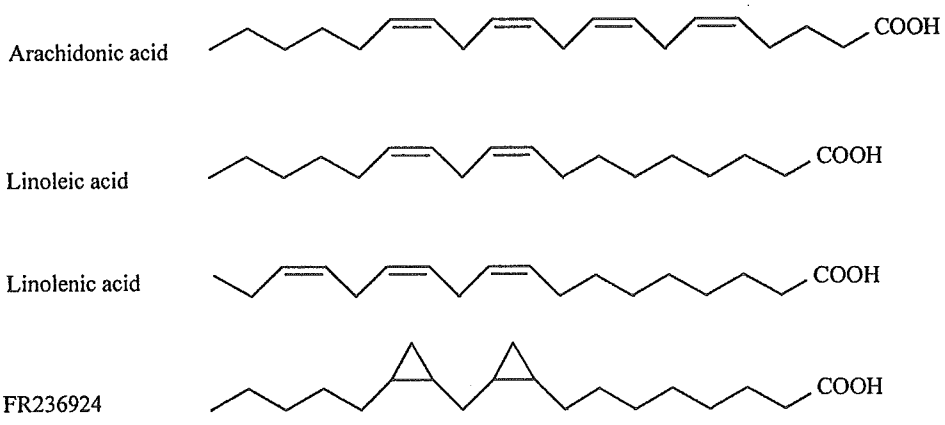


Fig. (24). Chemical structures of arachidonic acid, linoleic acid, linolenic acid and FR236924.

bonds. FR236924 (10 μmol/L) induced a gradually developing and persistent potentiation of α7 nAChR responses [152]. The effect of FR236924 was inhibited by the selective PKC inhibitor GF109203X or the α7 nAChR antagonist α-bungarotoxin [152]. Furthermore, it has been reported that FR236924 increased the rate of AMPA receptor-mediated miniature excitatory postsynaptic currents, without affecting the amplitude, triggered by nicotine in rat hippocampal CA1 pyramidal neurons, and that the effect could be inhibited by GF109203X or α-bungarotoxin [153]. FR236924 stimulated glutamate release from rat hippocampal slices and in the hippocampus of freely moving rats, and the effect was also inhibited by GF109203X or α-bungarotoxin [153]. These findings suggest that FR236924 stimulates glutamate release by functionally targeting presynaptic α7 nAChRs on the glutamatergic terminals under the influence of PKC, responsible for the facilitatory action on hippocampal synaptic transmission. It is thus possible that FR236924 would be a useful drug for studying the regulatory mechanisms underlying cognitive functions linked to *cis*-unsaturated free fatty acids and α7 nAChRs.

KYNURENIC ACID AS AN ENDOGENOUS ANTAGONIST OF α7 nAChR

Kynurenic acid is synthesized via kynurenine from the essential amino acid L-tryptophan, and kynurenic acid is produced and released by astrocytes in the brain [21,133,154,155] (Fig. 3). Interestingly, it has been reported that the levels of kynurenic acid are increased in the CSF and brain of schizophrenic patients [156,157]. In addition to its well-characterized action as a competitive antagonist of the glycine site on the NMDA receptors, kynurenic acid also acts as a noncompetitive antagonist of the α7 nAChRs [154,155] (Fig. 3). Administration of L-kynurenine, a precursor of kynurenic acid together with probenecid, an inhibitor of organic acid transport, increased levels (500-fold) of kynurenic acid in the hippocampus of rats, and also disrupted auditory sensory gating in rats. In contrast, administration of L-701,324, a centrally acting antagonist of the glycine site of NMDA receptors, failed to disrupt auditory gating in rats, suggesting that elevated levels of kynurenic acid produce the disruption in auditory processing through α7 nAChR [158].

D-Serine is synthesized from L-serine via serine racemase in astrocytes (Fig. 3). Glutamate binds to AMPA receptors on astrocytes that stimulate the release of D-serine. The released D-serine binds to glycine sites on NMDA receptors [20,21,159-161]. Several findings suggest that D-serine plays a role as an endogenous agonist on glycine sites of the NMDA receptors [20,21,159-161]. We reported that serum levels of D-serine are significantly reduced in patients with schizophrenia, supporting the NMDA receptor hypofunction in schizophrenia [19,162]. Furthermore, we found that the ratio of D-serine to total serine in the CSF of first episode and drug naive schizophrenic patients was significantly lower than that of age-matched normal controls [163]. These data suggest that the synthetic and/or metabolic pathway of D-serine may be impaired in the brains of schizophrenic patients [21,163]. It would thus seem that both kynurenic acid and D-serine may act as endogenous ligands at glycine sites of the NMDA receptor and thereby play a role in NMDA receptor hypofunction in schizophrenia [21] (Fig. 3).

Taken together, these results indicate that blockade of α7 nAChR in the hippocampus by elevated levels of kynurenic acid is likely to lead to auditory gating deficits in schizophrenic patients, and that disruption of reciprocal astrocyte-neuron signaling mechanisms involving kynurenic acid and D-serine may play a role in the pathophysiology of schizophrenia [21] (Fig. 3).

CONCLUDING REMARKS

As described above, α7 nAChRs play a role in the deficits of P50 auditory sensory gating in schizophrenia. Direct (α7 nAChR agonists) or indirect agonists (5-HT_3 receptor antagonists, positive allosteric modulators, FK960, FR236924, PNU-120596) of α7 nAChRs would be useful as potential therapeutic drugs for the treatment of schizophrenia, since deficits of P50 auditory sensory gating have been associated with the cognitive impairments in schizophrenia. Considering the role of kynurenic acid and D-serine as endogenous ligands at glycine sites of the NMDA receptors, it is possible that agents which could decrease the levels of kynurenic acid or increase the levels of D-serine in the brain would be possible therapeutic drugs for schizophrenia.

In addition, patients with Alzheimer's disease have also been reported to show deficits of P50 auditory sensory gating relative to controls [164]. It has also been suggested that the disturbed sensory gating in patients with Alzheimer's disease might result from cholinergic dysfunction or α7 nAChR loss. Furthermore, it has been shown that α7 nAChRs might be implicated in the disposition of β-amyloid as well as cognitive impairments in Alzheimer's disease [165,166]. Therefore, it is also likely that agonists of α7 nAChRs would be useful as potential therapeutic drugs for the treatment of Alzheimer's disease.

ABBREVIATIONS

ACh	=	Acetylcholine
GABA	=	γ-Aminobutyric acid
nAChR	=	Nicotinic acetylcholine receptor
CNS	=	Central nervous system
CSF	=	Cerebrospinal fluid
NMDA	=	N-methyl-D-aspartate
CHRNA7	=	α7 nAChR subunit gene
DMXB-A (GTS-21)	=	3-(2,4)-Dimethoxybenzilidine anabaseine
DMAB	=	3-(4)-Dimethylaminobenzylidine anabaseine
DMAC	=	3-(4)-Dimethylaminocinnamylidine
4-OH DMXB-A	=	3-(4-Hydroxy-2-methoxybenzylidine) anabaseine
AR-R17779	=	(-)-Spiro[1-azabicyclo[2.2.2]octane-3,5'-oxazolidin-2'-one]
SIB1765F	=	[±]-5-Ethynyl-3-(1-methyl-2-pyrrolidinyl)pyridine fumarate
W-56203	=	(R)-3'-(3-Methylbenzo[b]thiophen-5-yl)spiro[1-azabicyclo[2.2.2]octane-3,5'-oxazolidin]-2'-one
AMPA	=	α-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
MLA	=	Methyllycaconitine
TC-1698	=	2-(3-Pyridyl)-1-azabicyclo[3.2.2]nonane
PNU-282987	=	N-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]-4-chlorobenzamide hydrochloride
SSR180711A	=	4-Bromophenyl 1,4-diazabicyclo[3.2.2]nonane-4-carboxylate hydrochloride
PCP	=	Phencyclidine
PSAB-OFP	=	(R)-(-)-5'-Phenylspiro[1-azabicyclo[2.2.2] octane-3,2'-(3'-H)furo[2,3-b]pyridine
PNU-120596	=	1-(5-Chloro-2,4-dimethoxy-phenyl)-3-(5-methyl-isoxazol-3-yl)-urea
FK 960	=	N-(4-Acetyl-1-piperazinyl)-p-fluorobenzamide monohydrate
PKC	=	Protein kinase C
FR 236924	=	8-(2-((2-Pentylcyclopropan-1-yl)methyl)cyclopropyl)octanoic acid

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RESEARCH ARTICLE

Functional polymorphism of the NQO2 gene is associated with methamphetamine psychosis

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Abstract

Several lines of evidence suggest that genetic factors contribute to the vulnerability of drug abuse such as methamphetamine (MAP), and that dopamine-quinones produced by administration of MAP may be involved in the mechanism of MAP-related symptoms. The detoxification of quinones is catalyzed by a family of proteins designated as quinone oxidoreductases (NQOs). We analysed the polymorphisms of NQO1 and NQO2 genes to elucidate the association with genetic vulnerability to MAP abuse in Japan. The genotype and allele frequencies for the polymorphism (Pro187Ser) of the NQO1 gene did not differ between each subgroup of patients and controls. In contrast, the genotype frequency for the insertion/deletion (I/D) polymorphism in the promoter region of the NQO2 gene was a significant ($p = 0.038$) difference between patients with prolonged-type MAP psychosis and controls. This study suggests that the NQO2 gene polymorphism contributes to the aetiology of MAP-related psychosis in Japanese.

Introduction

Misuse of methamphetamine (MAP), the most abused drug in Japan, is a growing problem world-wide. Several lines of evidence suggest that genetic factors contribute to the vulnerability of substance abuse (Kendler 2001; Tsuang et al. 2001; Crabbe 2002; Uhl et al. 2002). Oxidative stress plays a role in the mechanisms of action of MAP: dopamine (DA) is released from the vesicular storage to the cytoplasm by administration of MAP, where DA can auto-oxidize to produce DA-quinones and additional reactive oxygen species, suggesting that DA-quinones might be implicated in the mechanism of action of MAP-induced neurotoxicity or psychosis in humans (Kita et al. 2003; Hashimoto et al. 2004).

The detoxification of quinones is catalyzed by a family of proteins designated as quinone oxidoreductase (NQOs). NQOs catalyze two-electron reduction to detoxify quinones competing with the one-electron reduction for the metabolism of quinones. In humans, genetic evidence indicates that the different forms of NQOs are encoded by four gene loci. Two of these gene loci have been identified as cytosolic NAD(P)H-quinone oxidoreductase 1 (NQO1) (Ross et al. 2000) and

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