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Smoking and schizophrenia: abnormal nicotinic receptor expression

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Abstract

Biological and genetic evidence suggests a role for the neuronal nicotinic receptors in the neuropathophysiology of schizophrenia. Nicotine normalizes an auditory evoked potential deficit seen in subjects who suffer from the disease. Nicotinic receptors with both high and low affinity for nicotine are decreased in postmortem brain of schizophrenics compared to control subjects. The chromosomal locus of the human α-7 gene (15q14) is linked to the gating deficit with a lod of 5.3, and antagonists of the α-7 receptor (α-bungarotoxin and methyllycaconitine) induce a loss of gating in rodents. We have cloned the human α-7 gene and found it to be partially duplicated proximal to the full-length gene. The duplication is expressed in both the brain and in peripheral blood cells of normal subjects, but is missing in some schizophrenic subjects. The results of these studies suggest the presence of abnormal expression and function of the neuronal nicotinic receptor gene family in schizophrenia.

Keywords: Nicotinic receptor; Schizophrenia; Nicotine; Smoking; Gene duplication; Neutrophil

1. Introduction

The incidence of smoking in the mentally ill, particularly in schizophrenia is much higher than in the general population. Nicotine normalizes a sensory deficit found in schizophrenia and both aberrant expression and regulation of nicotinic acetylcholine receptors have been found in postmortem brain, isolated from subjects who suffered from this disease in life. Over the last 10 years we have investigated the biology and genetics of nicotinic receptors in sensory processing deficits found in schizophrenia. Current progress continues to support the hypothesis that aberrant nicotinic receptor expression plays an important role in this common mental disorder.

2. Smoking in the mentally ill

A large number of studies suggest that the use of tobacco products among the mentally ill is significantly higher than in the general population and may be markedly higher than in normal subjects. As smoking declines in this country, the fraction of total smokers who are mentally ill increases. It is estimated that of the total smokers in the United States today, more than 25% are mentally ill (Leonard et al., 1998a,b,c)!

A comparison of smoking history in subjects from whom tissues have been collected in our local studies supports this hypothesis (Fig. 1A). Tissues collected include postmortem brain and peripheral blood from both schizophrenic and control subjects. We found that the incidence of smoking was higher in subjects suffering from depression and bipolar disorder and inordinately higher in schizophrenics. Interestingly, the concomitant use of alcohol and cigarettes in schizophrenia is markedly different from normal smokers and smokers suffering from

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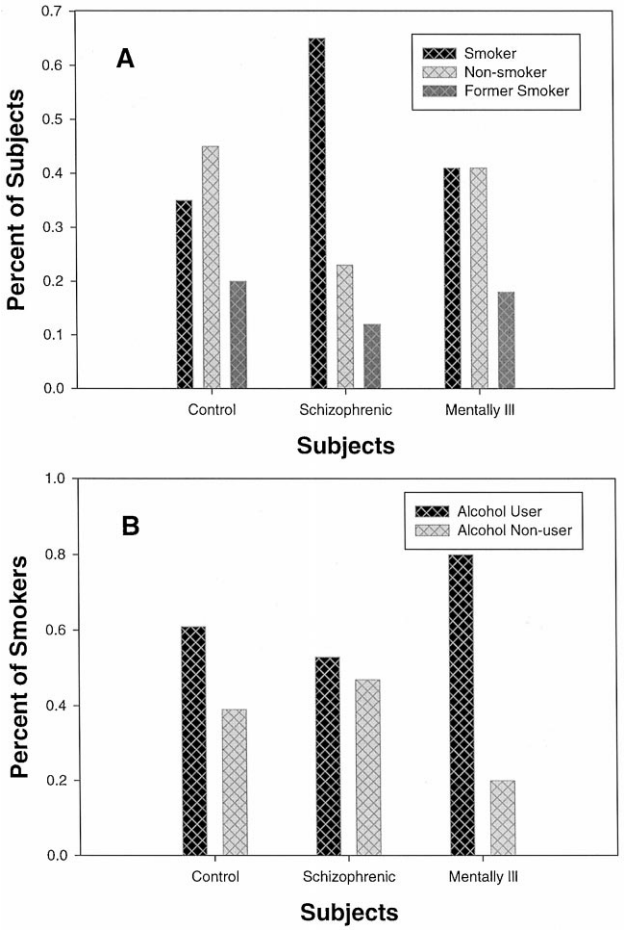

Fig. 1. Distribution of smoking histories and alcohol use in control and mentally ill subjects. Tissue includes human postmortem brain collected following donation by family members, and blood samples. Tissue was collected from control, schizophrenic and mentally ill subjects. The mentally ill group includes subjects with either depression or bipolar disorder. (A) Percent of total subjects collected who were smokers, non-smokers, or former smokers; (B) percent of smokers who were either users or non-users of alcohol.

other forms of mental illness. In subjects from whom we have collected tissues, we found that fewer schizophrenic smokers also used alcohol than subjects in either of the other groups (Fig. 1B). Although the reason for the lower incidence of alcohol use in schizophrenia than in the other populations is not known, the use of cigarettes in schizophrenia could have a different etiology than either depression or bipolar disorder.

The finding of increased smoking in schizophrenics is not a new one, as evidenced by studies of multiple investigators (Hughes et al., 1986; Greeman and McClellan, 1991; Menza et al., 1991; Goff et al., 1992; Brown et al., 1996).Schizophrenics appear to extract more nicotine from each cigarette than normal smokers, possibly due to different inhalation patterns (Olincy et al., 1997). The use of tobacco in the schizophrenic population can also be pathologic, including use of multiple forms of tobacco and development of water intoxication (Kirch et al., 1985).

The important question to be resolved is whether this highly significant incidence of smoking is biologically related to the disease in some way or whether it is the result of medications. Both pharmacological and genetic evidence supports an underlying biological relevance to the heavy use of tobacco products in schizophrenia.

3. Sensory processing deficits in schizophrenia

Schizophrenia is a common and complex disorder with diverse symptoms including auditory hallucinations, delusions and a flat affect (DSMIV). It affects approximately 1% of the population, worldwide, usually presents in early adulthood and generally affects the patient the rest of his life. Childhood onset schizophrenia, between the ages of 6 and 12, is also recognized (Jacobsen and Rapoport, 1998; Ross et al., 1999).

A strong genetic component for schizophrenia is well-documented (McGue and Gottesman, 1989; Tsuang and Faraone, 1994; Risch, 1990). The incidence is higher in family members than in the general population and monozygotic twins have a higher risk of developing the disorder if one twin has the disease than do fraternal twins. Genetic linkage studies have not been conclusive, resulting in confirmed linkage on multiple chromosomes (Risch and Merikangas, 1993; Cloninger, 1994). The chromosomal regions of linkage are large, making positional cloning and identification of candidate genes difficult. This has led to biological study of endophenotypes in the disease, where the genetics and neuronal pathways involved are likely to involve fewer genes. The idea is not a new one (Meehl, 1962; Venebles, 1964; Tsuang, 1993), and has led to the discovery of several sensory processing deficits in schizophrenics that can be studied in animal models where invasive pharmacology can be done.

Recent examples include auditory evoked potential and prepulse inhibition deficits, recorded as abnormally gated electrical responses in the brain (Adler et al., 1982; Braff and Saccuzzo, 1985; Braff et al., 1992) and deficits in eye tracking (McGlashan and Fenton, 1992; Tsuang, 1993). Multiple laboratories have confirmed that these traits are indeed abnormal in a majority of schizophrenics.

Auditory gating is measured in humans as a decremented response to the second of two consecutively administered auditory stimuli (Adler et al., 1982; Boutros et al., 1991; Freedman et al., 1991). In normal subjects, the amplitude of the second response is less than 40% of the conditioning response. In schizophrenics, the test to conditioning ratio (T/C) is almost always > 0.5 and can be even higher than the conditioning amplitude ($T/C \geq 1.0$). This gating deficit is found in $> 75\%$ of schizophrenic subjects and in 50% of family members, whether or not they have the disease (Waldo et al., 1991, 1995). Unlike schizophrenia, which does not have a Mendelian inheritance, the P50 deficit appears to be inherited as an autosomal dominant trait in schizophrenic families.

Interestingly, the P50 deficit is normalized by nicotine use in both schizophrenics and family members (Adler et al., 1992, 1993), suggesting that they may be attempting some type of self-medication (Adler et al., 1998). Another sensory deficit, in eye movement, is also normalized by nicotine. Anticipatory saccades, an abnormal response in the capacity to smoothly follow a moving visual target (Ross et al., 1998a,b), disappear following nicotine administration (Olincy et al., 1998). Bilineal inheritance of the eye-tracking disorder has been found in families of childhood onset schizophrenics (Ross et al., 1999).

4. Animal models of the auditory gating deficit

Animal models of sensory gating deficits have been useful for pharmacological experiments, resulting in the discovery that the α-7 nicotinic acetylcholine receptor is likely to play an important role in the inhibition of the second of paired auditory stimuli. The locus of the gated waveform in human brain has been localized to the temporal lobe (Goff et al., 1980; Reite et al., 1988a,b). Since it is not possible to perform invasive experiments to more precisely define the neuronal pathway in man, we have used a rodent model to study the pharmacology of this endophenotype. A similar gated wave can be recorded in the laboratory rat (P20) from both the CA3 region of the hippocampus (Bickford-Wimer et al., 1990) and in the septum (Miller and Freedman, 1993), indicating there may be some type of reciprocal control to and from the septal/hippocampal projection.

Antagonists of some nicotinic acetylcholine receptors block the filtering of auditory input in the rat. More specifically, only antagonists of a subtype of the nicotinic receptor family, containing α-7 subunits (Wonnacott, 1986), blocked auditory gating in an anesthetized rat model (Luntz-Leybman et al., 1992). Both the snake toxin α-bungarotoxin and a delphinium derivative, methyllycaconitine, specific antagonists of the α-7 nicotinic receptor, were potent blockers of the inhibitory response to the second tone in the awake behaving rat (Rollins et al., 1993; Leonard et al., 1996, 1998a,b,c). Antagonists of neither muscarinic acetylcholine receptors (scopalamine), nor the high affinity nicotinic receptors (mecamylamine), had an effect. In a more specific experiment, antisense oligonucleotides complementary to the translation start site of the α-7 receptor mRNA, blocked gating of the second of paired auditory stimuli in the awake behaving rat (Rollins et al. 1993; Leonard et al., 1996). The decrease in [^{125}I]α-bungarotoxin binding resulting from the antisense treatment was approximately 40%, suggesting that a decrease in α-7 receptor expression of this magnitude was sufficient to block the auditory gating response.

We have also studied the effects of nicotinic receptor agonists on auditory gating in both rats and mice. Agonists of the α-7 nicotinic receptor actually improve auditory gating. A comparison of effects of the α-7 receptor antagonist methyllycaconitine and a new α-7 partial agonist, 3-(2,4)-dimethoxybenzylidine anabaseine (GTS-21, DMXB-A) showed that methyllycaconitine significantly elevates the test to conditioning ratio, indicating loss of auditory gating, while GTS-21 significantly improves it (Leonard et al., 1998a). GTS-21 also normalizes aberrant auditory gating found in the DBA mouse strain, which has decreased levels of α-7 receptor expression (Stevens et al., 1996,1998). The agonist response was blocked by α-bungarotoxin, but not by mecamylamine (Stevens et al., 1998), suggesting that GTS-21 is acting specifically at the α-7 nicotinic receptor.

5. Expression of nicotinic receptors in schizophrenic brain tissue

The expression of both high and low affinity receptors has been compared in postmortem brain from schizophrenic and control subjects. In a receptor autoradiographic study using [^{125}I]α-bungarotoxin, low affinity receptors were found to be decreased in postmortem hippocampus of schizophrenics (Freedman et al., 1995) by approximately 50%. This ligand is known to bind to the low affinity nicotinic receptor subunit, α-7 (Clarke, 1992, 1993; Séguéla et al., 1993). Using [^{3}H]nicotine, we have recently found that high affinity nicotinic receptors are also decreased in several postmortem brain regions, including hippocampus, cortex, striatum and thalamus (Breese et al., 1999). Further, receptor numbers were not upregulated by smoking to the same extent as we had found in normal subjects (Breese et al., 1997). Although smoking appears to increase the metabolism of the typical neuroleptic haloperidol (McEvoy et al., 1995a), failure to upregulate receptors does not appear to be due to haloperidol use (Lee et al., 1999).

However, the effects of medication on nicotinic receptor numbers in schizophrenics do need to be further investigated. Clozapine seems to decrease the drive to smoke in schizophrenics who are using this medication (McEvoy et al., 1995b, 1999). Interestingly, clozapine normalizes the P50 deficit in schizophrenics (Nagamoto et al., 1996), an effect hypothesized to be blockade of 5-HT3 receptors with subsequent release of acetylcholine (Adler et al., 1998). Although haloperidol does not appear to alter expression of nicotinic receptors (Lee et al., 1999), it is not yet known whether clozapine has an effect.

6. Genetic evidence for involvement of nicotinic receptors in the auditory gating deficit in schizophrenia

Independent evidence of a role for the α-7 nicotinic receptor subunit in the P50 sensory processing deficit was shown when the chromosomal locus for α-7 on human

chromosome 15 was genetically linked to this auditory gating measure (Freedman et al., 1997). A polymorphic marker < 120 kb from the α-7 gene was linked to the P50 deficit with a lod score of 5.3, $\Theta = 0.0$. This finding has been replicated by our laboratory in a second cohort (Leonard et al., 1998c), and by two other groups (Riley et al., 1997; Kaufmann et al., 1998). The same locus has also recently been linked to bipolar disorder (Edenberg et al., 1997; Craddock and Lendon, 1999), suggesting that defects in the α-7 gene may be common to both schizophrenia and manic depression. Although high affinity nicotinic receptors appear to be decreased in schizophrenic postmortem brain, as well, there is presently no confirmed linkage to a locus for any known high affinity subunit.

The mouse model for the auditory gating deficit, the DBA strain, has been used for genetic studies. Polymorphisms in the murine α-7 gene in DBA mice have been linked to low levels of α-7 expression in this strain (Stitzel et al., 1996). Auditory gating deficits have been found to be inversely correlated with α-bungarotoxin binding in mice; the DBA mouse had the most aberrant gating deficit (Stevens et al., 1996). These results suggest that a common defect, resulting in decreased α-7 expression, may affect auditory gating in both man and mouse.

7. Cloning of the human α-7 gene and a partial duplication

The human α-7 gene has been cloned and found to have 10 exons (Gault et al., 1998). A partial duplication was isolated, mapping proximal to the full-length gene on chromosome 15q14. Exons 5–10 are duplicated with a cassette of approximately 1 Mb and inserted downstream of novel exons (Gault et al., 1998). The duplication is expressed as mRNA with these four novel exons (A–D). The novel exons are also duplicated within the cassette, leading to a complex genomic structure at this locus. The role of the expressed gene duplication remains to be explored, but it appears to be expressed as mRNA in both brain and in peripheral tissue.

A promoter for the human α-7 full-length gene has also been isolated. It is similar in structure to the bovine and chick α-7 gene promoters, including conservation of Sp-1 and Egr-1 consensus transcription factor binding sites (Couturier et al., 1990; Carrasco-Serrano et al., 1998). A cyclic-AMP responsive binding (CREB) site is present in the human gene that is not found in the chick (Gault et al., 1998).

8. Expression of nicotinic receptors in peripheral blood cells

Further examination of nicotinic receptor expression in schizophrenia will require a readily available tissue for quantification of receptor expression in family members. Nicotinic receptors have recently been found in many peripheral tissues, suggesting that they may have non-synaptic roles (Lukas et al., 1993; McGehee and Role, 1995). Developing muscle expresses α-7 mRNA (Romano et al., 1997), and most subunits have been found in small cell lung carcinomas and other tissues of apparent neural crest origin (Quik et al., 1994; Logel et al., 1997). Nicotinic receptors are also found in both lymphocytes and neutrophils in peripheral blood (Lebargy et al., 1996). Many cells in the periphery have both a cholinergic phenotype and nicotinic receptors on their surface (Wessler et al., 1998).

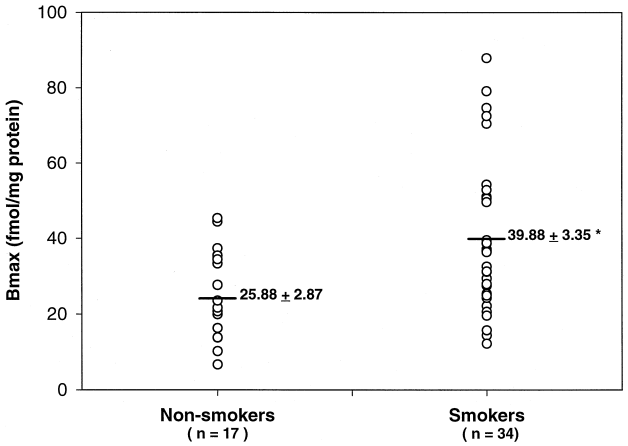

Fig. 2. Scatter plot of [^{3}H]nicotine binding levels in human peripheral blood neutrophils of non-smokers and smokers. Preparation of cellular membranes and ligand binding was previously described in Breese et al. (1997). Smokers had a significant increase in the number of nicotinic receptors, compared with the non-smokers (* $p < 0.009$).

We have developed an assay for [^{3}H]nicotine binding in peripheral blood cells, which are easily obtained from clinical subjects (Lebargy et al., 1996). We have found that nicotine binding in polymorphonuclear cells is increased in smokers (Fig. 2), as it is in the brain (Breese et al., 1997). Further, receptor number is correlated with the number of cigarettes smoked per day, suggesting that it will be a good correlate for nicotine binding studies in families (Benhammou et al., 1999). The assay in peripheral blood will be used to expand our results showing decreased levels of high affinity nicotinic receptors in schizophrenic brain tissue (Breese et al., 1999). Mutation screening of both the full-length and duplicated α-7 genes in schizophrenic subjects is in progress.

9. Concluding remarks

Both genetic and molecular biological studies of the nicotinic receptor gene family in schizophrenics continue to support a role for the α-7 receptor subunit in sensory processing defects seen in this disease. Recent progress suggests that high affinity nicotinic receptors may also be

involved. Assay of receptors in the peripheral blood, where levels reflect smoking history, will be an important tool for measurement of high affinity nicotinic receptor levels in schizophrenics and family members.

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