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Review

Genetic matters: Thirty years of progress using mouse models in nicotinic research

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ABSTRACT

This research update summarizes thirty years of studies on genetic influences on responses to the acute or chronic administration of nicotine. Early studies established that various inbred mice are differentially sensitive to the effects of the drug. Classical genetic analyses confirmed that nicotine effects on locomotion, body temperature and seizures are heritable. A significant inverse correlation between the locomotor and hypothermic effects and the density of nicotine binding sites suggested that differential expression α4β2-neuronal nicotinic acetylcholine receptor (nAChR) mediated some of this genetic variability. Subsequent studies with α4 and β2 nAChR null (decreased sensitivity) and gain of function mutants (increased sensitivity) supports the role of the α4β2*nAChR subtype. However, null mutant mice still respond to nicotine, indicating that other nAChR subtypes also mediate these responses. Mice differing in initial sensitivity to nicotine also differ in tolerance development following chronic treatment: those mice that are initially more sensitive to nicotine develop tolerance at lower treatment doses than less sensitive mice, indicating that tolerance is an adaptive response to the effects of nicotine. In contrast, the sensitivity of mice to pre-pulse inhibition of acoustic startle response is correlated with the expression of α7-nAChR. While genetic variability in nAChR expression and function is an important factor contributing to differences in response to nicotine, the observations that altered activity of opioid, glutamate, and cannabinoid receptors among others also change nicotine sensitivity reinforces the proposal that the genetics of nicotine response is more complex than differences in nAChRs.

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1. Introduction

Evidence for the importance of genetic factors in mediating tobacco use in humans was first provided by the R.A. Fisher in 1958 [1]. Since then many different approaches, including twin studies and more recently genome wide association studies have firmly established that genetic factors are important components in tobacco use in humans (see reviews [2–6]).

Our research has used mouse models to investigate the role of genetics in mediating responses to nicotine. A useful initial step to assess the role of genetic factors on any response is

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the characterization of variability among defined genetic populations. The laboratory mouse is an excellent resource with which to begin the evaluation of genetic factors because of the availability of a large number of inbred strains. More recently, the mouse has been the species widely used to generate genetically modified lines, mostly gene knockout and knockin lines. Tests of the roles of specific genes on responses of interest are now possible.

2. Locomotor activity and body temperature

2.1. Inbred mouse strains and classical genetic analysis

We initiated our studies on the role of genetic factors in mediating responses to nicotine using available inbred mouse strains. An early study examined the effect of an acute administration of nicotine by constructing full dose–response curves for several behavioral and physiological responses in four common inbred strains (BALB, C57BL/6, DBA/2 and C3H/IBG) [7]. Even with this fairly modest number of strains both quantitative differences (approximately a 4-fold difference in ED_{50} values for nicotine-induced hypothermia) and qualitative differences (locomotor depression in three of the inbred strains but locomotor activation in C3H mice in the open field arena) were observed. Certainly, genotype influenced response to nicotine in the mouse. However, with this limited number of mice a relationship between behavioral response and nicotinic receptor expression (measured with nicotine and α-bungarotoxin binding in tissue homogenates) could not be determined.

The observation of substantial strain differences in response to nicotine prompted two studies examining the heritability of these responses using a diallel cross. The parental strains for this analysis were the four strains screened initially (BALB, C57BL/6, DBA/2 and C3H/IBG) and A. All possible F1 hybrids were generated and tested for the effect of nicotine on hypothermia [8] and open-field activity [9]. Both analyses confirmed that strain differences exist and also demonstrated heritability of the nicotine-induced responses consistent with an additive/dominance model. A significant directional dominance toward increased sensitivity to nicotine that was particularly pronounced for the locomotor response was observed. That is, the hybrid mice were more sensitive to nicotine than predicted by the parental responses. This directional dominance was interpreted from an evolutionary point of view to be indicative of a selective advantage where increased sensitivity could protect against ingestion of toxic levels of nicotine.

The screen of inbred mice was subsequently expanded to include 19 strains [10]. A multi-component test battery was designed to allow the measurement of several different responses to nicotine in an individual mouse. The battery consisted of measurements of the effects of nicotine on respiratory rate, acoustic startle response, crosses and rears in the Y-maze, heart rate and body temperature. The efficiency of the test battery allowed construction of full nicotine dose–response curves for each strain. Substantial differences in ED_{50} values (4–5-fold for most tests) were observed among the strains, further establishing the importance of genetic factors in mediating nicotine-induced responses. Correlational analysis of the results revealed that the effects of nicotine on the activity measures and body temperature were very similar, a result confirmed by factor analysis. Overall these analyses indicated the existence of four groups of mice ranging from those that are very sensitive to nicotine (C57BL/10, C57BL/6 and A) to those that are very resistant (BUB and C58). Two additional subsets were also identified, one that is moderately sensitive (including DBA/1 and DBA/2) and a second that is moderately resistant (including C3H and CBA).

In order to investigate whether the variation in acute response to nicotine is a consequence of variability in expression of nicotinic receptors, the binding of nicotine and α-bungarotoxin was measured in homogenates prepared from eight different brain regions. It is now well established that nicotine labels α4β2*-nAChR sites [11,12] (the * represents the potential for additional subunits [13]) and α-bungarotoxin labels α7-nAChR sites [14]. A significant overall negative correlation between the density of nicotine binding sites and ED_{50} values for nicotine effects on activity and body temperature was observed [15]. The correlation between α-bungarotoxin binding and these ED_{50} values was not statistically significant. This result indicated that the density of α4β2-nAChR was inversely correlated with sensitivity to locomotor and hypothermic effects of nicotine: the higher α4β2*-nAChR expression, the lower the dose of nicotine necessary to elicit a response. However, these results should be and have been regarded as merely suggestive.

2.2. nAChR null, gain of function mutants and natural variants

With the development of genetically modified mice the nAChR can either be deleted (null mutants) or mutated to enhance agonist sensitivity (gain of function) (see [16] for review). Both types of mutants have been generated for the Chrna4 and Chrnb2 genes, which encode the α4 and β2 nAChR subunits, respectively. The availability of these genetically modified mice allows the direct test of the effects of altered α4β2-nAChR expression on response to acute nicotine administration. The results presented in Fig. 1 demonstrate the effect of deletion of either the α4 or β2 nAChR subunit gene or insertion of hyperactive α4 (L9′A) or β2 (V22′L) nAChR subunit gene on nicotine effects on Y-maze crosses or body temperature. Significant changes in sensitivity to acute nicotine injection were noted for mice from which wild-type versions of either the α4 or β2 nAChR subunits were deleted or replaced with a mutant hyperactive receptor subunit. Deletion of either α4 (new data) or β2 [17] resulted in a gene-dose dependent decrease in sensitivity to acute nicotine. In contrast, insertion of a hypersensitive version of either α4 (new data) or β2 [18] resulted in a gene-dose dependent increase in sensitivity to nicotine. It should be noted, that the null mutant mice still responded to acute nicotine administration illustrating that the α4β2-nAChR was not the only receptor subtype that regulates nicotine-induced hypomotility or hypothermia.

The studies described above concentrated on the relationship between the density of α4β2*-nAChR expression and response to nicotine. However, a polymorphism representing a single point mutation in the Chrna4 gene changed in the primary sequence of the subunit (ala/thr difference at position 529) and an alteration of receptor function [19]. This mutation was originally identified in the long sleep (LS) and short sleep (SS) mice that were selected for their differential sensitivity to ethanol and also differ in response to nicotine [20]. Recombinant inbred (RI) strains generated by inbreeding mice isolated from a F2 cross of these mice were tested for their responses to acute nicotine administration. RI mice differing in the Chrna4 polymorphism were also differentially sensitive to nicotine-induced hypomotility and hypothermia [21]. In heterologous expression systems, α4β2-nAChR can assemble with two alternative stoichiometries [$(\alpha4)_2(\beta2)_3$ with high agonist sensitivity to agonists (HS form) and $(\alpha4)_3(\beta2)_2$ with lower agonist sensitivity (LS)] [22–25]. These alternate stoichiometries are also found in mouse brain [26,27]. The observation that the A529 T polymorphism affects the relative expression of the two alternative stoichiometric forms of the α4β2-nAChR receptor with intrinsic differences in sensitivity to activation by nicotinic agonists [28] suggests that this and perhaps other point mutations in a receptor subunit can alter nicotine responses by changing the