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Association between genetic variability of neuronal nitric oxide synthase and sensorimotor gating in humans

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ABSTRACT

Research increasingly suggests that nitric oxide (NO) plays a role in the pathogenesis of schizophrenia. One important line of evidence comes from genetic studies, which have repeatedly detected an association between the neuronal isoform of nitric oxide synthase (nNOS or NOS1) and schizophrenia. However, the pathogenetic pathways linking nNOS, NO, and the disorder remain poorly understood. A deficit in sensorimotor gating is considered to importantly contribute to core schizophrenia symptoms such as psychotic disorganization and thought disturbance. We selected three candidate nNOS polymorphisms (Ex1f-VNTR, rs6490121 and rs41279104), associated with schizophrenia and cognition in previous studies, and tested their association with the efficiency of sensorimotor gating in healthy human adults. We found that risk variants of Ex1f-VNTR and rs6490121 (but not rs41279104) were associated with a weaker prepulse inhibition (PPI) of the acoustic startle reflex, a standard measure of sensorimotor gating. Furthermore, the effect of presence of risk variants in Ex1f-VNTR and rs6490121 was additive: PPI linearly decreased with increasing number of risk alleles, being highest in participants with no risk allele, while lowest in individuals who carry three risk alleles. Our findings indicate that NO is involved in the regulation of sensorimotor gating, and highlight one possible pathogenetic mechanism for NO playing a role in the development of schizophrenia psychosis.

1. Introduction

Schizophrenia is a severe and chronic mental disorder with devastating consequences for patients and their relatives. Due to its high prevalence, affecting about 1% of the population worldwide, schizophrenia also represents a serious public health problem (for a review, see e.g. Ref. [1]). Research increasingly suggests that nitric oxide (NO) might be importantly involved in schizophrenia pathogenesis. NO plays a role in several processes implicated in schizophrenia such as brain development, neuronal plasticity, regulation of neurotransmission and oxidation-reduction balance (for a review, see e.g. Ref. [2]). In neurons, NO is produced by neuronal isoform of nitric oxide synthase (nNOS or NOS1) as a consequence of the activation of glutamate N-methyl-D-aspartate (NMDA) receptors and the subsequent calcium influx (for reviews, see e.g. Refs. [3,4]). Reduced synthesis of NO and resulting insufficient activation of its effector pathways (such as reduced activity of soluble guanylate cyclase and cGMP formation) may thus represent an important component of the compromised signaling via NMDA receptors, considered to play a major role in schizophrenia (for a review, see e.g. Ref. [5]). It is well established that inhibition of NMDA receptors elicits acute symptoms of schizophrenia [6], and a randomized double-blind placebo-controlled trial has shown that administration of NO donor nitroprusside rapidly improved severity of symptoms in schizophrenia patients [7]. A number of studies have found altered levels of nNOS expression or NO metabolites in postmortem brain tissue of patients with schizophrenia (for reviews, see Refs. [8–10]). Several studies reported that specific variants of the nNOS gene occur more often in schizophrenia patients, suggesting nNOS as a candidate gene for schizophrenia (for reviews, see Refs. [9,11–13]).

The risk for developing schizophrenia mostly depends on genetic

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susceptibility, but the number of contributing genes is high and the impact of individual risk variants is small (for recent reviews, see Refs. [14,15]). Given the plentitude of risk genes and the complexity of the schizophrenia phenotype, one promising approach is to focus on intermediate phenotypes (also termed endophenotypes), which are more closely related to the neurobiological underpinnings of the disorder [16]. One important schizophrenia endophenotype is sensorimotor gating, which refers to an automatic process of filtering out irrelevant stimuli so that behavior can be directed toward the significant aspects of the environment [17]. Disrupted gating may thus contribute to psychotic disorganization and thought disturbance [18–20]. Sensorimotor gating is typically assessed by measuring the prepulse inhibition of the acoustic startle reflex (PPI), which denotes a reduction of the startle response to a loud auditory stimulus when it is shortly preceded by a weak non-startling stimulus (for a recent review, see Ref. [21]). PPI is decreased in schizophrenia patients, but also in healthy individuals at increased genetic risk of developing the disorder such as first-degree relatives [22]. Association with PPI was reported for several schizophrenia candidate genes, indicating shared genetic roots of gating deficits and schizophrenia [23–26].

Here we report a study in which we tested whether the efficiency of sensorimotor gating is related to genetic variability of nNOS. For this purpose, we specifically selected three candidate nNOS polymorphisms: i) a VNTR (variable number of tandem repeats) polymorphism in the promoter region of exon 1f (Ex1f-VNTR), ii) a SNP (single nucleotide polymorphism) of exon 1c (rs41279104, hereafter termed Ex1c-SNP), and iii) a SNP in intron 10 (rs6490121, hereafter termed In10-SNP) [27–30]. These polymorphisms were specifically chosen based on their reported association with schizophrenia, as well as with executive functions in healthy individuals. We hypothesized that individuals who carry the alleles previously identified as risk variants will display a decreased PPI. Moreover, given that a significant genetic contribution to both PPI and schizophrenia may be attributed to a cumulative effect of common risk variants [26,31–34], we also expected that PPI will be lower in individuals carrying a higher number of nNOS risk alleles.

2. Material and methods

Here we only briefly describe the procedures and methods used in this study. For details please refer to the Supplementary Information.

A sample of 128 subjects was screened for inclusion in the study. Screening was focused on medical history, psychopathological status and hearing ability. Drug consumption (including nicotine) was determined by a urine toxicology test. Ninety-six healthy adults with no first-degree relatives with a mental disorder met the criteria and participated in the study (mean age ± SD = 24.2 ± 3.5 years, 80 males, 33 smokers). An a priori power analysis for ANCOVA model including one fixed factor (2–3 groups) and one covariate indicated that the sample size of the study was sensitive enough to detect effect sizes equal to or larger than $\eta_p^2 = 0.07$ to 0.10 ($\alpha = 0.05$, $1 - \beta = 0.80$, G*Power [35], which corresponds to the effect sizes reported in previous genetic studies of PPI [36–39]. In accordance with the Declaration of Helsinki, all participants gave informed consent with study participation. The study was approved by the Ethics Committee of the Institute of Normal and Pathological Physiology, Slovak Academy of Sciences (approval number EK/2/16).

The experimental session began with a 3-min acclimation period to a background white noise of 55 dB, which was continuously presented throughout the session. The paradigm comprised six separate stimulus conditions: pulse alone (PA) trials and five different types of prepulse-pulse (PP) trials. In PA trials, white noise pulses at an intensity of 105 dB, and a duration of 40 ms with instantaneous rise/fall time were presented. In PP trials, pulses were preceded by prepulses: white noise at an intensity of 75 dB, and a duration of 20 ms with instantaneous rise/fall time. In PP trials, five different PP intervals were used: 30, 60, 120, 2000 or 4000 ms. The experimental session consisted of 69 trials, which were divided into three blocks. Block #1 consisted of five successive PA trials. In block #2 ten PA trials and ten PP trials for each PP interval were administered in random order. The paradigm ended with block #3, consisting of four PA trials. The inter-trial interval was randomly selected on each trial from an interval from 10 to 20 s.

The startle response was assessed following the guidelines by Blumenthal et al. [40]. An electromyogram (EMG) of periocular muscles was measured bilaterally, analog filtered (1–1000 Hz) and sampled at 2048 Hz. Further offline processing included digital filtering (28–800 Hz bandpass, 48–52 Hz notch), epoching (from −100 to 400 ms with respect to startle stimulus onset), and removal of epochs containing artifacts. The magnitude of the startle response was measured as the peak value of the rectified EMG waveform within a time window ranging from 21 to 150 ms after stimulus onset. The response was considered as absent if the amplitude within 21–120 ms did not exceed the mean of the baseline interval (0–20 ms) by at least 2 standard deviations. Mean blink amplitudes were calculated for each subject and trial type. Data from the left and the right eye were averaged. Conditions with PP intervals 2000 and 4000 ms were excluded from the analysis (since they use to elicit prepulse facilitation). Prepulse inhibition at PP intervals 30, 60 and 120 ms (hereafter denoted as PPI30, PPI60 and PPI120) was calculated according to the formula $(1 - \text{mPP}/\text{mPA}) \times 100\%$, where mPP and mPA denote mean startle amplitude in PP and PA trials respectively. Baseline startle reactivity (BSR) was assessed as the mean startle response in block #1 and was used as a covariate in the statistical tests of PPI [41].

Material for genotyping was obtained from buccal mucosa swabs. Genotyping was carried out using standard procedures (for details see Supplementary Information). Alleles of nNOS Ex1f-VNTR polymorphism were classified as short (19–27 GT repeats) or long (28–36 GT repeats) in accordance with earlier studies [42]. All genotypes were distributed according to Hardy-Weinberg equilibrium and linkage disequilibrium was weak (Supplementary Tables 1 and 2). The genotype groups did not differ in demographic characteristics (Supplementary Tables 4-6).

3. Results

A significant PPI was present at all tested PP conditions (mean PPI30 = 64.6%, 95% CI [60.8–68.5%]; PPI60 = 74.0%, [71.0–76.9%]; PPI120 = 72.5%, [69.6–76.2%]). The effects of genotype on PPI were tested using a repeated measures analysis of covariance (ANCOVA) with PP interval as a within-subjects factor, BSR was included as a covariate due to its positive association with PPI (PPI30: $r = 0.238$, PP60: $r = 0.472$, PPI120, $r = 0.465$). The analysis revealed statistically significant effects of Ex1f-VNTR and In10-SNP, which both were independent of the PP interval (Table 1). Effects of Ex1c-SNP on PPI were not statistically significant. The effects of genotype did not change with the inclusion of age, sex and smoking status as covariates to the statistical models (additional details are provided in Supplementary Tables 3-6).

Next, given the significant effects of Ex1f-VNTR and In10-SNP on PPI, these two polymorphisms were analyzed for additive effects. For this purpose, subjects were grouped by the total number of risk alleles carried at the given loci (from 0 to 3; the only individual with four risk alleles was excluded). Using a linear mixed-effect model (LMEM), PPI was modeled as a function of the number of risk alleles (Risk), controlling for BSR and PP interval, and a random effect Subject. The interclass correlation coefficient was large ($ICC = .859$) suggesting that PPI values from the different PP conditions were not independent and confirming that LMEM with random intercepts (Subject) was appropriate. The model showed that Risk significantly affected PPI, $F(1,92) = 7.428$, $p = .008$, $R^2 = 0.074$, with each additional risk allele decreasing PPI by 3.76% (SE = 1.38). As expected, the control variables also significantly predicted PPI (PP interval: $F(2,186) = 3.162$,