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Upregulation of adenosine A2A receptors induced by atypical antipsychotics and its correlation with sensory gating in schizophrenia patients

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Abstract

Sensory gating deficits have been found in patients with schizophrenia and their unaffected relatives. However, the underlying neurobiological mechanism of this deficit remains unclear. Pre-clinical studies have implicated adenosine in sensory gating deficits in schizophrenia. Therefore, the current study investigated a possible relationship between peripheral adenosine A2A receptor (ADORA2A) and sensory gating indices (P50 measures) in medication-free schizophrenia (n = 31) and healthy (n = 21) groups. The effects of six-week antipsychotic treatment were examined. At baseline, schizophrenia patients showed impaired sensory gating compared to healthy controls. However, there was no significant difference in ADORA2A gene expression among groups. In addition, ADORA2A expression was not correlated with sensory gating at any time point. Following treatment, we found a significant upregulation of ADORA2A expression. Intriguingly, we observed a significant positive association between ADORA2A upregulation and baseline P50 amplitudes in the schizophrenia group. A main finding of the current pilot study is the upregulation of ADORA2A expression following treatment with antipsychotics. In addition, this

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Contributors

Chongtao Xu, Jie Zhang, and Renhua Wu designed the study and wrote the first draft of the paper. Xiaohong Hong and Qingjun Huang reviewed and revised the manuscript. Junqing Wang, Chunlian Liang, Yuzhen Liu, and Haixing Huang performed clinical interviews and rated the patients. Jie Zhang conducted the real-time PCR and analyzed the data. Junqing Wang, Jie Zhang, Xiaona Wan, Bilan Wen, and Liyun Jiang administered the P50 paradigms to patients and analyzed the data. Chadi G. Abdallah contributed to the analysis of the data, the interpretation of the findings and to the writing of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

The authors report no conflicts of interest concerning this study.

upregulation was predicted by baseline P50 amplitude, an observation that awaits replication in an expanded sample.

Keywords

sensory gating; antipsychotic; adenosine; schizophrenia

1. Introduction

The ability of the human brain to filter out irrelevant stimuli that occur in close temporal proximity is essential for selection, processing, and storage of information arising from the outside world (Alho, 1992; Cadenhead et al., 2000). “Sensory gating”, as traditionally measured by P50 event-related potential (ERP), refers to the ability of the brain to inhibit incoming irrelevant sensory input (Freedman et al., 1991), and has been extensively used to measure basic inhibitory processes in schizophrenia (Adler et al., 1991; Adler et al., 1998; Boutros et al., 2004; Bramon et al., 2004). Recent meta-analyses confirmed previously reported sensory gating deficits in patients with schizophrenia (de Wilde et al., 2007; Patterson et al., 2008; Thaker, 2008). Moreover, sensory gating deficits have been proposed as an endophenotypical marker, particularly as the deficit also occurs among unaffected relatives of patients with schizophrenia (Adler et al., 1999; Freedman et al., 2003; Hall et al., 2011). Hence, in the current study we attempted to investigate the underlying neurobiological mechanisms of this sensory gating deficit.

Accumulating evidence indicate that P50 suppression is regulated by a multistep process and wide-ranging neural circuitry, prominently involving hippocampal structures (Wilson et al., 1984; Waldo et al., 1994), and other brain regions, such as the temporoparietal and prefrontal cortices. The neurophysiological basis of P50 suppression is not yet completely understood (Hershman et al., 1995; Adler et al., 1998; Light et al., 1999). Antipsychotic treatment, which primarily affect the dopaminergic system, did not significantly alter the deficits of P50 gating in schizophrenia (Freedman et al., 1983). While the cholinergic system could regulate some of these gating deficits through stimulation of $\alpha 7$ -nicotinic receptors (Adler et al., 1993), the activation of $\alpha 7$ -nicotinic receptors from human brain interneurons failed to trigger GABAergic postsynaptic currents that are implicated in P50 suppression (Akhondzadeh et al., 2000). These findings suggested that P50 suppression could be regulated by other modulators of synaptic activity, such as adenosine.

Adenosine is an important neuromodulator in the human CNS, exerting potent inhibitory influences on synaptic activity (Brundege et al., 1997), predominately through activation of distinct high affinity A1 and A2A receptor subtypes (Cunha, 2001). Recently, convergent evidence implicated adenosine in the neuropathology of schizophrenia (Lara et al., 2000; Lara et al., 2006; for recent review see Boison et al., 2011). Dysfunction of adenosine A1 receptors, which have a profound effect on brain development (Rivkees et al., 2001), was proposed to underlie the two-hit hypothesis of schizophrenia, accounting for the neurodevelopmental aspect of this disorder (Rivkees, 1995; Dumas et al., 1998; Othman et al., 2003; Back et al., 2006). Another receptor of interest is adenosine A2A receptor (ADORA2A), which co-localizes with dopamine D2 receptors on GABAergic interneurons and forms a functional complex with opposing effects (Ferre, 1997; Hillion et al., 2002). Thus, it is suggested that the increased basal D2 receptor occupancy by dopamine in schizophrenia patients is correlated with decreased adenosinergic receptors activity (Ferre, 1997; Svenningsson et al., 1999). Consistent with this hypothesis, acute administration of high doses of caffeine, a nonselective adenosine receptors antagonist, to schizophrenia patients exacerbates their positive symptoms (Lucas et al., 1990). Moreover, increased

striatal ADORA2A expression has been found in postmortem studies of patients with schizophrenia, further implicating dysregulation of ADORA2A receptors in the pathology of schizophrenia (Kurumaji et al., 1998; Deckert et al., 2003). Despite these preliminary findings, mostly derived from pre-clinical and animal studies, there is limited data on the adenosine systems directly obtained from patients with schizophrenia.

In relation to the underlying mechanism of sensory gating deficits in schizophrenia, adenosine has been shown to influence neurophysiological responses of P50-evoked potential (Lara, 2002). In addition, an elegant study showed that an activity-dependent release of adenosine from hippocampal slices produced heterosynaptic inhibition similar in pattern to a P50-evoked paradigms (Mitchell et al., 1993). Moreover, theophylline and caffeine can impair sensory gating measured with P50 paradigm in healthy subjects, resembling the phenotype observed in patients with schizophrenia (Ghisolfi et al., 2002; Ghisolfi et al., 2006). Of relevance to ADORA2A receptors, mice lacking this receptor showed impaired sensory gating as measured by prepulse inhibition (PPI) of the startle reflex (Nagel et al., 2003; Wang et al., 2003). Additionally, conditioning (S1) response – a P50 measure – is regulated by dopamine, thus ADORA2A deficits could precipitate dopamine D2 receptors hyperactivity and subsequently sensory gating impairment as measured by P50 paradigm (Ghisolfi et al., 2006). This close association between dopamine activity and ADORA2A expression and function has been recently supported by *in-vitro* studies (Trincavelli et al., 2011). In spite of these indirect findings on the possible relationship between adenosine and sensory gating, direct evidence defining a relevant role for adenosine in the inhibitory deficits has yet to be provided. ADORA2A is the only adenosine receptor expressed peripherally on the platelets surface (Varani et al., 1999). In addition, ADORA2A and D2 dopamine receptors, assembled into heteromeric complexes, are co-expressed on the human platelets and regulated by treatment with antipsychotic medication in patients with bipolar disorder (Martini et al., 2006; Trincavelli et al., 2010; Trincavelli et al., 2012), which suggested that peripheral ADORA2A can be utilized as a surrogate to investigate adenosine impairment in schizophrenia.

Therefore, in the present study, we determined P50 measures and ADORA2A gene expression at baseline in a medication-free schizophrenia group and in a well-matched healthy controls group. These measures were repeated following six-week treatment with atypical antipsychotics. This allowed us to investigate differences among groups at baseline and following treatment. In addition, we examined correlations between sensory gating, ADORA2A, and severity of symptom in patients with schizophrenia.

2. Materials and Methods

2.1. Subjects

We evaluated 33 patients with schizophrenia and 21 healthy controls. Participants' gender, age, diagnosis, and years of education are summarized in Table 1. Two patients dropped out before study completion, with 31 patients completing the study and included in further analyses. All patients were recruited from the Mental Health Center of Shantou University. Each patient was diagnosed with either schizophrenia or schizophreniform disorder by an experienced psychiatrist using the Structured Clinical Interview for the DSM-IV (SCID) (First, 2002). Those with schizophreniform diagnosis were followed up for at least six months to confirm a diagnosis of schizophrenia. The exclusion criteria involved cardiovascular or neurological disease, a history of head injury that resulted in a loss of consciousness, or meeting criteria for DSM-IV Axis I mood or anxiety disorders. All patients were either psychotropic-naïve (no prior treatment with psychotropic medications) or had been medication-free for at least one month before the sensory gating measurement and detection of ADORA2A gene expression. The controls were healthy volunteers

recruited from among the staff of Shantou University and the local community. All of the subjects had normal hearing acuity and signed informed consent before participating in the study. Sensory gating measurements and detection of ADORA2A gene expression for the patient group were obtained before they received antipsychotics (pre-treatment) and after six weeks of antipsychotic treatment (post-treatment). ADORA2A gene expression and P50 measures were determined at baseline and following the treatment period, except for post-treatment P50 measures that were not tested in the healthy group. The schizophrenia group received antipsychotic treatment, including olanzapine ($n=10$, range 10-20 mg/day, mean $\pm SD$ 16 $\pm$ 3.94 mg/day, chlorpromazine-equivalent doses of 320 $\pm$ 24.6 mg/day), risperidone ($n = 9$, range 4-7 mg/day, mean $\pm SD$ 5.0 $\pm$ 1.0 mg/day, chlorpromazine-equivalent doses of 250.00 $\pm$ 50.00 mg/day), quetiapine ($n = 7$, range 450-700 mg/day, mean $\pm SD$ 571.43 $\pm$ 69.86 mg/day, chlorpromazine-equivalent doses of 761.91 $\pm$ 93.15 mg/day), aripiprazole ($n = 5$, range 10-20 mg/day, mean $\pm SD$ 25 $\pm$ 7.07 mg/day, chlorpromazine-equivalent doses of 240 $\pm$ 22.1 mg/day).

2.2. P50 testing

2.2.1. Apparatus and Procedures—The electrophysiological examinations were performed, at the Laboratory of Clinical Neurophysiology at the Shantou University Mental Health Center, using a signal generator and the data acquisition system of a digital 40-channel EBNeuro Sirius BB BE. All subjects were asked to abstain from smoking and tea drinking for at least 30 min prior to testing. Testing took place in a quiet, lighted room that was not electrically shielded. Eye movements were recorded via electro-oculography (EOG) with Ag/AgCl disc electrodes placed at the outer canthus and below the right eye. Recordings were obtained with a disc electrode affixed to the vertex (CZ site) and referenced to left temporal apophysis. All electrode resistances were less than 5 K Ω . The stimuli were generated by means of computer-driven, 90-dB pulses of 0.1 msec in duration produced by a signal generator, and a data acquisition system was used to record the ERP waveforms. After acquisition of the data, those trials that contained artifacts (± 50 uv wave amplitude deviation) were not included in the wave form averaging. Data were analyzed offline, and band-pass filtered (10-50Hz, 12dB/octave roll-off).

2.2.2. Evoked Potential Paradigms—Stimuli were presented as a series of auditory click pairs. The interval between click pairs was 10 seconds. At least three sets of averaged evoked responses to 32 pairs of stimuli were obtained from subjects at inter-stimulus intervals (ISIs) of 500 msec between the conditioning stimulus (S1) and test stimulus (S2). The ERP responses were amplified and band-pass filtered with a 0.1 to 300 Hz analog filter and 50-Hz notch filter.

2.2.3. P50 component measurement—The conditioning-testing paradigm P50 component was identified as described in previous reports (Nagamoto et al., 1989; Sanchez-Morla et al., 2008). P50 was defined as the most positive deflection 40 to 90 msec after stimulus presentation. The response to the S2 test stimulus selected was the most positive wave in the latency range equal to the latency of the S1 response $\pm$ 10 msec. P50 amplitude was defined as the absolute difference between the P50 peak and the preceding negative trough. The evoked potential peaks, amplitudes, and latencies were first screened with an automated computer algorithm and then manually verified off-line by investigators blind to subject and condition. P50 gating was defined as P50 ratios (amplitude S2/amplitude S1), and to minimize skewed distributions, ratios greater than one were assigned the value one (Smith et al., 1994).

2.3. Q-RT-PCR detection of gene expression of platelet ADORA2A

2.3.1. Platelet purification—According to the modified centrifugation, filtration, and leukocyte depletion protocol (Bugert et al., 2003; McRedmond et al., 2004), human platelets were purified from 5 ml blood collected from each participant and immediately were held in test tubes containing 0.5 ml sodium citrate. Platelet-rich plasma (PRP) was obtained through centrifugation (15 min, 150×g at room temperature (RT)). Platelets were pelleted from the PRP at 800×g for 10 min and resuspended in a few ml of washing liquid for platelets (148 mM NaCl, 0.6 mM EDTA2Na, 5 mM glucose, 20 mM Tris, pH 7.4). Platelets were then purified, as previously described, by gradient centrifugation through a 25–35% sucrose gradient (Soslau et al., 1982). After leukocyte depletion, platelets were collected by centrifugation (10 min, 800×g, RT), dissolved in 1 mL TRIzol® (Invitrogen, CA, USA) and stored at –85 °C until RNA isolation.

2.3.2. RNA isolation, cDNA generation and Q-RT-PCR assessment—Total platelet RNA was isolated using TRIzol according to the manufacturer's instructions (TRIZOL® Reagent Cat. No. 15596-018) with the following modifications: after phase separation, the upper aqueous phase was incubated at –20 overnight with 10 µg glycogen (Invitrogen) and centrifuged for 15 min at 12,000×g, 4. Each individual RNA pellet was dissolved in 25 µl RNase-free water and stored at –85 .

PCR primers were designed using the Primer Express 2.0 software program (Perkin-Elmer Applied Biosystems; Foster City, CA). The cells and their primers are summarized in Table S.1 (online supplement). Two-step RT-PCR was performed. Specifically, 1 µg of total RNA was reverse transcribed in a total volume of 20 µl using the Reverse Transcription System cDNA synthesis kit (Promega BioSciences, San Luis Obispo, USA). This RT reaction was conducted at 42 for 15 min, 95 for 5 min and terminated at 0 for 5 min. TaqMan real-time quantitative PCR amplification reactions were carried out in an AB 7300 Real-Time PCR system (Applied Biosystems, Foster City, USA) using TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, USA) with 5 µl of cDNA plus 12.5 µl 2× TaqMan Buffer in a total volume of 25 µl. PCR was performed at 94 for 10 min followed by 40 cycles at 94 for 30 s, 60 for 30 s, 72 for 60 s.

2.3.3. Quantitation of ADORA2A gene expression—The delta-delta method was used as described by Perkin-Elmer Applied Biosystems to determine the relative levels of mRNA expression between experimental samples and controls, as reported previously (Livak and Schmittgen, 2001; Pfaffl, 2001). Briefly, it assumes that the real-time PCR achieves optimal amplification efficiencies (*E*) for both the target and reference genes. Under these conditions we can define CT as the threshold cycle value of the PCR reaction and use this to calculate the amplification ratio = $2^{-(\Delta CT_{\text{sample}} - \Delta CT_{\text{control}})}$, which can be further simplified as the ratio = $2^{-\Delta\Delta CT}$. This CT value is inversely proportional to the logarithmic scale of the starting quantity of template DNA. Therefore, samples containing low concentrations of target molecules require more PCR cycles to amplify enough copies to reach the detected threshold amplification.

2.4. Statistical analysis

All statistical analyses were performed using SPSS software (SPSS 19.0 for Mac OS X 10.xx). Prior to each analysis, the normality of outcome measures was examined using Shapiro-Wilk test. Consequently, nonparametric tests were used for skewed distributions. First, schizophrenia and healthy groups measures were compared using independent t-test and Mann-Whitney U. Then, changes over the treatment periods were tested using paired t-test and related-samples Wilcoxon Signed Rank test. PANSS scores improvement were computed by subtracting baseline minus final scores. ADORA2A gene expression and P50

gating ratio changes were computed by subtracting final minus baseline measures. Finally, correlations were investigated using Pearson and Spearman tests. All tests were two-tailed, with significance level set at $p = 0.05$.

3. Results

3.1. Schizophrenia vs. Healthy Groups

The two groups did not differ in terms of age ($p = 0.58$), gender ($p = 0.52$), education ($p = 0.14$), or proportion of smokers ($p = 0.78$). At baseline, schizophrenia patients showed impaired P50 sensory gating relative to healthy controls. Test amplitudes (S2) in schizophrenia group were higher than in healthy. In addition, conditioning amplitudes (S1) were lower in the schizophrenia group as compared to healthy controls (see Table 2). This impairment was particularly evident on P50 gating ratio measure, which showed high P50 ratio in schizophrenia group (Table 2). There were no differences in latencies between groups (Table 2).

3.2. Antipsychotic Effect on Sensory Gating and PANSS Scores

Examining the effect of antipsychotic treatment on sensory gating in the schizophrenia group showed no statistically significant effect on all P50 measures except for test amplitude (S2), where there was a significant reduction following treatment ($p = 0.04$; see Table S.2 – online supplement). Conversely, antipsychotic treatment exerted a potent effect on all PANSS measures, showing considerable PANSS scores reduction over the treatment period (see Fig. S.1 – online supplement). PANSS scores did not correlate with P50 gating ratio at baseline ($p > 0.50$). Similarly, following treatment, there were no associations between PANSS scores and P50 gating ratio ($p > 0.25$) – except for a positive correlation between PANSS-Positive scores and P50 gating ratio ($r_{\text{Spearman}} = 0.38$, $p = 0.03$).

3.3. Antipsychotic Effect on Adenosine A2A Receptor Expression

At baseline, peripheral ADORA2A gene expression was not significantly different between schizophrenia and healthy groups ($p = 0.40$; see Fig 1). However, the difference reached significance following six weeks of treatment, after which the schizophrenia group had higher ADORA2A gene expression (Mean $\pm$ SD = 1562 ± 1370) as compared to healthy controls (Mean $\pm$ SD = 888 ± 901 ; $T = 2.66$, $df = 50$, $p = 0.008$). This difference was further explored by examining the direct effect of antipsychotic treatment on ADORA2A using Related-samples Wilcoxon Signed Rank test. This analysis showed a significant effect of treatment on ADORA2A gene expression in the schizophrenia group ($p = 0.003$). Accordingly, there were no significant changes in ADORA2A gene expression in the healthy group ($p = 0.96$; see Fig. 1).

In the schizophrenia group, correlation analyses showed no association between changes in ADORA2A gene expression over the treatment period and changes in P50 gating ratio ($p = 0.38$). However, ADORA2A gene expression increase, following treatment, was positively correlated with baseline test (S2) amplitude ($r_{\text{Spearman}} = 0.61$, $p < 0.001$) and conditioning (S1) amplitude ($r_{\text{Spearman}} = 0.50$, $p = 0.004$). There were no correlations with other P50 measures. In addition, there was no correlation between changes in ADORA2A gene expression and PANSS scores, except for a negative association with improvement in PANSS-Positive scores ($r_{\text{Spearman}} = -0.36$, $p = 0.050$).

4. Discussion

In a medication-free group, we replicated previous findings of impaired P50 gating in schizophrenia (Table. 2). In addition, we found a limited effect of antipsychotic treatment on

sensory gating, with minimal, yet statistically significant, reduction of S2 amplitude but no other changes in P50 measures (Table S.2). Additionally, antipsychotics exerted a potent clinical effect, with considerable reduction on PANSS scores (Fig. S.1). Most importantly, we found a robust effect of antipsychotic on the expression of peripheral ADORA2A gene, revealing an upregulation following 6 weeks of treatment (Fig. 1). Finally, there were no consistent correlations between ADORA2A, P50 measures, and PANSS scores at any time point. Intriguingly, we observed a significant positive association between ADORA2A upregulation and baseline S1 and S2 amplitudes in the schizophrenia group, which suggests that baseline conditioning and testing responses predicted ADORA2A responses to antipsychotic treatment in this cohort.

In conditioning-test paradigms, higher test (S2) wave amplitudes were related to impaired P50 gating ratios in schizophrenia. This is consistent with previous findings of sensory gating impairments in patients with schizophrenia, which may reflect a deficit in filtering or gating in response to sensory stimuli, accounting for a disability to organize and integrate outside information (Freedman et al., 1991; Erwin et al., 1998; Brockhaus-Dumke et al., 2008). Moreover, results presented here are in line with previous reports of P50 suppression deficits in first-episode schizophrenia patients, suggesting that sensory gating deficits are possible endophenotypes of a genetic defect in schizophrenia (Bramon et al., 2004; Hong et al., 2009). The P50 wave amplitude is thought to reflect the brain's inhibitory gating effect, which mainly determined by reduced test (S2) wave amplitude in conditioning-test paradigms (Erwin et al., 1991). Consistent with previous findings, we found that treatment with antipsychotics has limited effect on P50 gating deficits (Erwin et al., 1994; Light et al., 2000; Adler et al., 2004; Hong et al., 2009). Of note, an elegant study by Hong et al. showed an advantage for theta-alpha-band gating as compared to traditional P50 gating (Hong et al., 2008), therefore, it is plausible that future studies employing theta-alpha gating measure would provide enhanced sensitivity to detect potential association with ADORA2A expression.

It has been hypothesized that a hypoadenosinergic state may attribute to the pathophysiology of schizophrenia (Kurumaji and Toru, 1998; Lara et al., 2000; Lara et al., 2006). However, whether hypofunction of ADORA2A is a cause or a consequence of hyperdopaminergic function remains unclear (Parsons et al., 1995; Johansson et al., 1997; Deckert et al., 2003). Moreover, the ability of caffeine to exacerbate positive symptoms of schizophrenia and the use of dipyrindamole – which inhibits adenosine degradation – for treatment of schizophrenia suggest that ADORA2A, combined with ADORA1, contribute to a hypoadenosinergic state in schizophrenia (Mikkelsen, 1978; De Freitas et al., 1979; Mayo et al., 1993; Akhondzadeh et al., 2000).

In the present study, increased levels in ADORA2A gene expression on platelets were found in schizophrenia subjects after receiving six weeks of antipsychotic medication, which is in agreement with previous findings (Parsons et al., 1995; Martini et al., 2006). In line with this result, a postmortem study of the striatum of patients with schizophrenia indicated that up-regulation of ADORA2A was correlated with prior antipsychotic treatment (Deckert et al., 2003). Yet, another postmortem study found increased density of ADORA2A in the striatum of schizophrenia patients regardless of antipsychotic treatment history (Kurumaji and Toru, 1998). The following reasons could account for this contradiction. ADORA2A-D2 receptor interaction in the basal ganglia can regulate the affinity of receptors for their respective neurotransmitters (Ferre 1997; Svenningsson et al., 1999). Most antipsychotic, especially typical antipsychotics, exert their effects primarily through blocking dopamine D2 receptor, suggesting that elevated levels of ADORA2A could be secondary to a lowered affinity of dopamine D2 receptors. However, atypical antipsychotics, can enhance its antipsychotic effects through stimulating striatal ecto-5'-nucleotidase activity that could increase the level

of adenosine and strengthen the function of ADORA2A (Pinna et al., 1999; Lara et al., 2001). Moreover, given the effect of atypical antipsychotics on non-dopaminergic neurotransmitters, such as glutamate and serotonin, adenosine's interaction with these neurotransmitters could account for its antipsychotic effects (Brunstein et al., 2001). Overall, our study suggests that increased peripheral ADORA2A expression in patients with schizophrenia is induced by atypical antipsychotic treatment.

While early studies suggested an involvement of adenosinergic neurotransmission in the development of the P50 inhibitory response (Mitchell et al., 1993; Ghisolfi et al., 2002; Ghisolfi et al., 2006), our study of peripheral ADORA2A gene expression failed to show consistent correlation with P50 measures. Compared to the wide expression of ADORA1 in the human brain, ADORA2A is highly expressed and localized in the basal ganglia. Thus, ADORA2A could have limited contribution to P50 suppression that primarily involves hippocampal structures, temporoparietal region, and prefrontal cortex (Wood et al., 1980; Waldo et al., 1994). In addition, other neural structures, such as cholinergic, noradrenergic, and GABAergic systems are involved in P50 gating procedure (Nagamoto et al., 1991; Adler et al., 1998). Finally, preliminary evidence of adenosine involvement in P50 inhibition was merely obtained from healthy subjects and pre-clinical studies (Mitchell et al., 1993; Ghisolfi et al., 2006), which further complicate any direct comparison with our findings.

The limitations of the current study include a small sample size and the varied use of different atypical antipsychotics. In addition, peripheral ADORA2A gene expression might not reflect the overall involvement of adenosine in the pathophysiology of schizophrenia. Finally, the strengths of the current study include a medication-free schizophrenia group along with a well-matched healthy controls, with a longitudinal design.

In conclusion, our study replicates previous findings of impaired sensory gating in schizophrenia. However, it failed to show aberrant ADORA2A level at baseline or consistent correlation with sensory gating. Perhaps, the major finding of this pilot study is the robust upregulation of peripheral ADORA2A expression in a cohort of medication-free patients following treatment with antipsychotics. In addition, the positive association between baseline P50 amplitude and ADORA2A upregulation is an intriguing observation that awaits replication in an expanded sample.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. The Effect of Antipsychotic Treatment on Adenosine A2A (ADORA2A) Receptor Gene Expression

At baseline, ADORA2A gene expression did not differ between groups. However, this difference reached significance following treatment. In addition, six-week of antipsychotic treatment exerted a considerable upregulation of ADORA2A gene expression in the schizophrenia group (Mean $\pm$ SD = 832 $\pm$ 521 to 1562 $\pm$ 1370, $p = 0.003$). There was no significant change of ADORA2A gene expression in the healthy group over a comparable six-week period ($p = 0.96$). Related-samples Wilcoxon Signed Rank test was used (significance set at $p = 0.05$).

Table 1

Baseline demographics and clinical dimensions of schizophrenia ($n = 31$) and healthy ($n = 21$) groups.

	Schizophrenia ^a	Healthy ^a
Age	24.00 ± 6.25	24.67 ± 1.68
Male	21	16
Smokers	13	12
Education (years)	9.61 ± 3.70	8.24 ± 2.47
Antipsychotics-naïve	8	
Subtypes		
Paranoid	19	
Undifferentiated	9	
Others	3	
Duration of illness (month)	12.11 ± 13.50	
PANSS score		
Positive Symptoms	26.2 ± 8.6	
Negative Symptoms	24.7 ± 7.7	
General Psychopathology	46.8 ± 8.7	
Total	103.8 ± 12.6	

^a Mean ± Standard Deviation are provided.

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Table 2

Comparison of sensory gating (conditioning-test paradigms) between medication-free schizophrenia patients and healthy controls

P50 Amplitude (µV)					
Conditioning	1.9 ± 0.9	2.9 ± 1.2	50	3.43	0.001
Test	1.6 ± 0.9	0.8 ± 0.4	50	3.28	0.001
P50 Latency (msec)					
Conditioning	56.1 ± 10.8	55.5 ± 6.5	50	0.25	0.81
Test	57.1 ± 12.5	54.3 ± 7.7	50	0.40	0.70
P50 Gating Ratio	0.76 ± 26	0.27 ± 13	50	5.12	<0.001

^aTest statistics from independent t-test or Mann-Whitney U.

^bIndependent t-test or Mann-Whitney U test (significance set at *p* = 0.05)