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Reduced Platelet Serotonergic Responsivity as Assessed by Dense Granule Secretion in First-Episode Psychosis

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SUMMARY

Objectives—To determine whether blunted serotonergic responsivity, indicated by decreased platelet dense granule secretion (DGS), occurs in neuroleptic-naïve patients with schizophrenia, as observed previously in chronic schizophrenia.

Design and Methods—Serotonin (5-HT)-amplified DGS was examined in 40 first-episode neuroleptic-naïve patients (24 with schizophrenia and 16 with mood disorders) and 24 healthy subjects.

Results—Healthy controls showed robustly increased DGS. Schizophrenic patients showed very modest DGS increases; mood disorder patients showed intermediate response.

Conclusions—Blunted DGS appears to be a characteristic of schizophrenia that is observed in the treatment-naïve condition.

INTRODUCTION

With the advent of atypical (serotonin-dopamine antagonist) antipsychotic agents there has been increasing interest in examining serotonergic system in schizophrenia. In addition to the role of 5-HT in a broad range of vegetative functions (appetite control, sexual behaviour, sleep regulation), 5-HT is involved in aggression, anxiety, depression, and psychosis. These functions and behaviours are mediated by 15 or more receptors, classified into seven groups: 5-HT₁ through 5-HT₇, with each group being further subdivided into receptor subgroups.

The propensity of platelets to respond to specific agonists, such as 5-hydroxytryptamine (5-HT), by shape change, dense granule secretion (DGS) and aggregation has been exploited to examine aspects of the serotonergic system. Similarities between platelet and brain 5-HT uptake sites provide validity to this approach. Dense granules, which contain serotonin, adenosine diphosphate (ADP) and calcium, are important for homeostasis and are released when platelets are stimulated by a variety of agonists such as ADP, collagen, thrombin, arachidonic acid, etc. In human platelets, 5-HT amplifies DGS stimulated by ADP (1), which

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is mediated by the 5-HT₂ receptor complex. Thus, the magnitude of serotonin amplification of ADP-induced DGS may be useful in evaluating the platelet serotonin responsivity.

DGS, along with assessment of platelet aggregation, has been utilized to examine platelet serotonergic responsivity in patients with chronic schizophrenia. These studies indicate that patients with chronic schizophrenia have blunted agonist-mediated platelet serotonergic responsivity, both during treatment and drug-free conditions (2,3). However, illness chronicity and treatment effects cannot be excluded from these studies. Examination of platelet serotonergic responsivity in first-episode neuroleptic-naïve patients, however, permits studies free from the confounding effects of treatment. Although DGS has been examined only in schizophrenia, alterations of other indices of platelet serotonergic responsivity have been found in some studies of patients with unipolar depression (4), raising the question of whether blunted serotonergic responsivity is specific to schizophrenia. In the present study a psychiatric control group was utilized to allow examination of this issue.

METHODS

Patients were recruited at first episode of psychosis, primarily in the inpatient setting, if they provisionally met DSM-IV criteria for schizophrenia and related psychotic disorders (schizophrenia, schizophreniform, schizoaffective disorder), or mood disorders with psychotic features based on Structured Clinical Interview for DSM Disorders (SCID). Initial and follow-up diagnoses are confirmed at a diagnostic conference attended by research faculty and staff, chaired by one of authors (MSK). Normal control subjects were recruited through local advertisement. We excluded subjects with previous exposure to antipsychotic agents, substance abuse during the preceding 6 months, systemic medical illness requiring treatment and neurological disorders, and in the case of normal subjects, a personal or family history of psychosis. Written informed consent was obtained from patients and normal control subjects after full explanation of the study. Blood samples were obtained in patients immediately prior to the initiation of antipsychotic agents. The Brief Psychiatric Rating Scale (BPRS) was administered at neuroleptic-naïve baseline, blind to any information regarding the biochemical assays.

Forty patients participated in the study. Twenty-four patients met DSM-IV criteria of schizophrenic disorder (17 male, 7 female) and sixteen patients with major depressive disorder with psychotic features (10 male, 6 female) served as the psychiatric control group. Twenty-four normal control subjects (17 male, 7 female) were recruited via advertisement in the local community. The mean age of patients with schizophrenia was 27.8 (SD 7.8) years, psychiatric controls 24.3 (SD 6.8) years, and healthy controls 27.0 (SD 8.0) years. The onset of psychosis in patients was at a mean age of 24.8 (SD 8.6) years. The years of education was significantly lower in patients (13.3±2.7 years) than control subjects (15.6±4.0 years).

Assessment of dense granule secretion

The method used to determine the platelet serotonin responsivity has been described previously (3). 450 µl of freshly drawn whole blood was diluted with 450 µl of saline in a cuvette containing a disposable siliconized magnetic stirrer. The sample was placed into the Chrono-Log Lumi-Aggregometer (model 500VS) at 37°C and spun at 1000 rpm. To each sample, 100 µl of Luciferin (0.16 mg/mL) was added and allowed to equilibrate. Luminescence of a 2 nmol scale was established by adding 5 µl of ATP standard solution (containing 2 nmol ATP) to the first sample prepared as above. Platelet DGS was stimulated with ADP (5.0 µmol/L) with and without 5-HT, and measured by comparing the change in luminescence in these samples to the 2 nmol ATP standard. ATP secretion was measured with a Dell PC model 5166GXMT interfaced with an AGGRO/LINK (Chrono-log Corp.).

Statistical analyses

Group means for the ADP-induced dense granule secretion was compared using one way analysis of variance (ANOVA). Serotonin responsivity was further evaluated by pairwise comparisons with two-tailed paired *t*-tests between baseline (ADP alone) and synergistic effect (ADP plus 5-HT). Spearman Rank correlation was used to determine whether platelet DGS was correlated with age, age at onset of illness and illness severity.

RESULTS

Healthy control subjects showed robust and highly significant increases in DGS in response to synergistic effect of ADP+5HT relative to ADP alone (79% and 74% increase at 0.2ug and 1.0ug 5-HT, respectively; $p < 0.001$ for both) (Table 1). By contrast, patients diagnosed with schizophrenia showed a modest, nonsignificant increase in DGS in response to 5HT (24% and 13% increase at 0.2ug and 1.0ug 5-HT, respectively; $p > 0.05$ for both). Patients diagnosed with mood disorders showed intermediate response (45% and 86% increase at 0.2ug and 1.0ug 5-HT, respectively; $p > 0.1$, and < 0.01 , respectively) (Table 1). There were no relations between platelet aggregation with age, age at onset of illness and illness severity as assessed by BPRS.

DISCUSSION

The present findings, albeit in a relatively small sample size, show that normal subjects had an expected robust response to ADP in a model of 5-HT-amplified DGS, while patients with schizophrenia did not. Patients with mood disorders, on the other hand, had about half (46%) the increase in DGS with ADP + 0.2ug 5-HT, relative to healthy controls (81%), but a significant and robust increase with 1.0ug 5-HT. The findings in this study for treatment-naïve patients with schizophrenia are consistent with the previously observed reductions in 5-HT-amplified DGS in patients with chronic schizophrenia (2), suggesting that treatment effects do not account for blunted serotonergic responsivity.

The findings of this study are also consistent with the observation that platelet aggregation (clumping), another index of platelet responsivity, is decreased in response to ADP and further amplification by 5-HT (Yao et al 1994; 1996). DGS is important to the process of platelet aggregation, and altered DGS consequently can alter platelet aggregation (5).

The relevance of these findings to understanding the pathophysiology of the serotonergic system in schizophrenia, and perhaps for mood disorders, remains to be fully elucidated. Platelets are produced in the bone marrow by megakaryocytes, with a lifespan of 7-10 days. The platelet membrane consists of a phospholipid bilayer and incorporates a series of receptor glycoproteins. The cytoplasm contains actin and myosin fibrils, as well three types of storage granules (dense granules, alpha granules, and glycogen granules). DGS can be initiated by a variety of agonists for which there are receptors, primarily G-coupled, in the platelet membrane. Previous studies have shown that different agonists (collagen, thrombin, arachidonic acid, 5-HT, etc) induce DGS differentially. In fact, DGS induced by collagen, thrombin and arachidonic acid is not different between healthy controls and patients (2). Only ADP and ADP + 5-HT induced DGS is different between patients and healthy controls. Thus, it is plausible that alterations in ADP and/or 5-HT receptors underlie the blunted DGS response in patients. ADP-induced platelet activation requires G-coupled receptor activation, involving Gq and Gi-coupled receptors, as well as the ADP P2Y1 or the 5-HT_{2A} receptors (6), and the degree of activation correlated with 5-HT₂ receptor density (1). Alterations in 5-HT₂ receptor density, both increased and decreased, have been reported in the literature (7). 5-HT receptor binding capacity depends on membrane fluidity, which have been found to be decreased in patient with schizophrenia (8). Membrane fluidity, in turn, is a function of membrane composition,

primarily the unsaturated fatty acids in membrane phospholipids. Reductions in membrane polyunsaturated fatty acids, particularly the ω -3 and ω -6 series have found in treatment-naïve and chronically-treated patients with schizophrenia by several investigators (9). It is noteworthy that oral supplementation with eicosapentaenoic acid, an ω -3 PUFA, in schizophrenia results in markedly enhanced 5-HT amplified platelet aggregation (10).

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Table 1

Serotonin-amplified platelet DGS by ADP Stimulation.

Subjects	(A) ADP alone	(B) ADP + 0.2 µg 5-HT	(C) ADP + 1.0 µg 5-HT	p (two-tailed paired t tests) (A) vs (B)	(A) vs (C)
Released ATP (nmoles):					
Normal controls	0.19 ± 0.15	0.34 ± 0.24	0.33 ± 0.28	< 0.0001	0.0058
Psychiatric Controls	0.22 ± 0.18	0.32 ± 0.23	0.41 ± 0.25	0.1051	0.0064
Schizophrenia	0.38 ± 0.39	0.47 ± 0.45	0.43 ± 0.46	0.0913	0.4301

Note. ADP, adenosine diphosphate; 5-HT, Serotonin; ATP, adenosine triphosphate