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# Reduced expression of G protein-coupled receptor kinases in schizophrenia but not in schizoaffective disorder

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ABSTRACT

Alterations of multiple G protein-mediated signaling pathways are detected in schizophrenia. G protein-coupled receptor kinases (GRKs) and arrestins terminate signaling by G protein-coupled receptors exerting a powerful influence on receptor functions. Modifications of arrestin and/or GRKs expression may contribute to schizophrenia pathology. Cortical expression of arrestins and GRKs was measured postmortem in control and subjects with schizophrenia or schizoaffective disorder. Additionally, arrestin/GRK expression was determined in elderly patients with schizophrenia and age-matched control. Patients with schizophrenia, but not schizoaffective disorder, displayed a reduced concentration of arrestin and GRK mRNAs and GRK3 protein. Arrestins and GRK significantly decreased with age. In elderly patients, GRK6 was reduced, with other GRKs and arrestins unchanged. A reduced cortical concentration of GRKs in schizophrenia (resembling that in aging) may result in altered G protein-dependent signaling, thus contributing to prefrontal deficits in schizophrenia. The data suggest distinct molecular mechanisms underlying schizophrenia and schizoaffective disorder.



## Introduction

Schizophrenia is a severe mental illness afflicting about 1% of the population. The dysadaptations of cortical G protein-coupled receptors (GPCRs) in schizophrenia has been the focus of many studies. The up-regulation of D1 receptors in the dorsolateral prefrontal cortex (Abi-Dargham et al., 2002) and abnormalities of D2-like receptors in the cortex in schizophrenia have been reported (Goldsmith et al., 1997; Schmauss, 1996). Postmortem studies consistently found changes in the cortical serotonin receptors, and many antipsychotic drugs target these receptors (reviewed in Abi-Dargham, 2007). Complex subtype- and brain region-specific changes in muscarinic receptors have also been reported (Scarr and Dean, 2008). Thus, the functional state of multiple cortical GPCR subtypes is modified in schizophrenia, although molecular mechanisms or functional consequences of these modifications remain unknown.

Signaling by GPCRs is controlled by multiple desensitization mechanisms. The main desensitization pathway involves activation-dependent receptor phosphorylation by G protein-coupled receptor kinases (GRKs) followed by arrestin binding that blocks G protein-mediated signaling (reviewed in Gainetdinov et al., 2004; Gurevich and Gurevich, 2003, 2006). Two arrestin subtypes, arrestin2 and arrestin3, and four GRK subtypes, GRK2, 3, 5, and 6, are ubiquitously expressed in the brain, each with a distinct distribution pattern (Arriza et al., 1992; Attramadal et al., 1992; Benovic and Gomez, 1993; Bezard et al., 2005; Gurevich et al., 2002, 2004; Premont et al., 1994). The functional specificity of arrestins and GRK is a matter of controversy. Although arrestin and GRK isoforms interact with many GPCRs *in vitro*, studies *in vivo* suggest that receptors are preferentially regulated by specific GRKs (Eckhart et al., 2000; Gainetdinov et al., 1999; Iaccarino et al., 1998a,b; Iwata et al., 2005; Koch et al., 1995; Luo et al., 2008; Naik et al., 2005; Rockman et al., 1996) and arrestins (Bohn et al., 1999, 2000, 2003; Kohout et al., 2001; Oakley et al., 2000). The signaling via GPCRs depends heavily on the cellular concentration of arrestins and GRKs, which in turn is influenced by the receptor usage (Ahmed et al., 2008a,b, 2010; Gainetdinov et al., 1999, 2003, 2004).

Here we tested the hypothesis that schizophrenia is associated with changes in the expression of arrestin and GRK isoforms in cortical areas implicated in schizophrenia pathology. The dorsolateral prefrontal cortex was the focus of the study, since an impressive body of evidence demonstrates deficits in this region in schizophrenia (reviewed in Lewis and González-Burgos, 2008). We have investigated the expression of arrestins and GRKs in the prefrontal cortex of patients with schizophrenia in comparison with patients with schizoaffective disorder and normal control, to assess the contribution of mood disturbance in addition to psychosis. Additionally, we have

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**Table 1**
Characteristics of subjects in the Harvard cohort.

| Diagnosis | Sex | Age, years | PMI, hours | 28S:18S RNA ratio | Substance abuse | Psychotropic medication/dose (mg/day) (antipsychotic drugs are underlined) | Cause of death |
|---|---|---|---|---|---|---|---|
| SA[a] | M | 52 | 14.8 | 1.2 | Alcohol | Fluphenazine decanoate 25 mg biweekly; Divalproex—500; Mirtazapine—30; Benztropine—2 | Cardiac arrest |
| SA | F | 49 | 23 | 1.29 | None noted | Haloperidol—10; Clozapine—150 | Pneumonia |
| SA | M | 48 | 7.6 | 1.47 | None noted | Quetapin—100; Divalproex—500; Venlafaxine—37.5; Benztropine—2 | Pneumonia |
| SA | M | 58 | 8.3 | 1.8 | None noted | Haloperidol—10; Artane—2 | Cardiac arrest |
| SA | M | 29 | 4.8 | 1.6 | None noted | Mesoridazine—350 | **Suicide (jump)** |
| SA | M | 46 | 10.5 | 1.78 | None noted | Haloperidol—19; Chlorpromazine—600; Thioridazine—100; Alprazolam—0.25; Phenytoin—300. | Sudden fatal cardiac arrhythmia followed by cardiorespiratory arrest |
| SA | M | 24 | 10 | 1.48 | None noted | Clozapine—400 | **Suicide by fire** |
| **N = 7** | **6M + 1F** | **43.7 ± 4.7**[c] | **11.3 ± 2.7**[d] | **1.52 ± 0.09**[e, f] | | | |
| SZ[b] | M | 32 | 7.8 | 1.22 | Cannabis, alcohol, marijuana | Clozapine—150; Divalproex—500; Clonazepam—0.5 | Acute pancreatitis |
| SZ | M | 68 | 21.4 | 1.31 | None noted | Quetapin—100; Aripiprazole—20; Divalproex—1000 | Cardiac arrest |
| SZ | M | 36 | 18 | 1.25 | None noted | Clozapine—350; Fluoxetine—60 | **Suicide by overdose** |
| SZ | F | 83 | 9.3 | 1.45 | None noted | Trifluopenazine—10; Sertraline—75 | Cerebral vascular aneurism |
| SZ | M | 47 | 17.8 | 1.42 | None noted | Quetapin—700; Sertraline—100 | Multiple myeloma |
| SZ | M | 64 | 15.4 | 1.2 | None noted | Fluphenazine decanoate—25 | Cardiac arrest |
| SZ | F | 56 | 18.7 | 1.71 | None noted | Olanzapine—10; Divalproex—500; Lorazepam—0.5 | Lung cancer |
| SZ | F | 48 | 22 | 1.53 | None noted | Risperidone—3; Olanzapine—5; Clozapine—400; Divalproex—500; Fluoxetine—60; Clonazepam—1 | Sudden death at home, cardiorespiratory in nature, natural |
| SZ | M | 42 | 10.3 | 1.58 | None noted | Quetapin—200; Haloperidol decanoate—100 mg, last injections—20 days before death | Homicide (gunshot) |
| **N = 9** | **6 M + 3 F** | **52.9 ± 5.5** | **15.6 ± 1.7** | **1.41 ± 0.06** | | | |
| Control | M | 69 | 21.1 | 1.41 | None | None | Myocardial infarction |
| Control | F | 53 | 16.6 | 1.29 | None | None | Cardiac arrest |
| Control | F | 86 | 6.9 | 1.59 | None | None | Myocardial infarction |
| Control | F | 51 | 23.1 | 1.42 | None | None | No cause of death |
| Control | M | 52 | 13.1 | 1.83 | None | None | No cause of death |
| Control | M | 24 | 21.3 | 1.4 | None | None | Cardiac arrest |
| Control | M | 61 | 10.1 | 1.26 | None | None | Myocardial infarction |
| Control | M | 28 | 23.3 | 1.6 | None | None | Unwitnessed cardiac arrest at home |
| Control | M | 48 | 12.1 | 1.56 | None | None | Myocardial infarction |
| Control | M | 24 | 5 | 1.92 | None | None | Homicide (gunshot) |
| **N = 10** | **7 M + 3 F** | **49.6 ± 6.4** | **15.3 ± 2.1** | **1.53 ± 0.07** | | | |

[a] SA—schizo-affective disorder.
[b] SZ—schizophrenia.
[c] There are no significant differences in Age among groups ($F(2,23) = 0.57$, $p = 0.572$).
[d] There are no significant differences in PMI among groups ($F(2,23) = 1.195$, $p = 0.32$).
[e] There are no significant differences in 28S:18S ratio among groups ($F(2,23) = 0.924$, $p = 0.41$).
[f] There is a tendency to reduced 28S:18S ratio with increased PMI across groups, but no significant correlation ($r = -0.352$, $p = 0.0778$) and no significant correlation between 28S:18S ratio and age ($r = -0.147$, $p = 0.48$).