

## Validating Novel Targets for Pharmacological Interventions in Schizophrenia

An unexpected hint from a rat model of psychosis led scientists to propose a new strategy to treat schizophrenia. In 1998, Bitá Moghaddam and Barbara W. Adams (1) observed that activation of group II metabotropic glutamate receptors (mGluRs) by LY354740 (an Eli Lilly agonist of mGluR2/mGluR3) in rats injected with phencyclidine attenuated the disruptive effects of phencyclidine on working memory, stereotypy, locomotion, and cortical glutamate efflux. This behavioral reversal occurred in spite of sustained dopamine hyperactivity. The finding opened a new approach to research on the potential treatments of psychiatric disorders that has extended the range of possible therapeutic targets beyond the dopamine hypothesis of schizophrenia. Almost a decade later, this discovery was followed by a clinical report (2) showing that agonists of group II mGluRs (LY404039 administered as the prodrug LY2140023) improved positive symptoms in patients with schizophrenia, with effects similar in magnitude to those of standard olanzapine treatment.

Glutamate, the major excitatory neurotransmitter in the brain, exerts fast effects by regulating ion flow, through AMPA, *N*-methyl-D-aspartic acid (NMDA), and kainate receptors, and modulatory effects by acting on different subtypes of G-protein coupled mGluRs. Converging lines of evidence implicate altered glutamatergic neurotransmission in the pathophysiology of schizophrenia (3). For example, exposure to NMDA receptor antagonists, such as phencyclidine or ketamine, produces “schizophrenia-like” symptoms in healthy individuals and exacerbates pre-existing symptoms in patients with schizophrenia (4). Such NMDA receptor antagonists are thought to mimic an intrinsic “hypofunction” of NMDA receptors on inhibitory  $\gamma$ -aminobutyric acid (GABA) neurons in schizophrenia, which leads to reduced inhibition of excitatory neurons and a consequent deleterious excess of glutamate release that disrupts the coordinated activity of neuronal networks (5, Figure 1). Because activation of the mGluR2 subtype of mGluRs, which is located on the terminals of excitatory axons, suppresses glutamate release, mGluR2 agonists were predicted in a clinical trial (6) to be beneficial in counteracting the consequences of the hypothesized “NMDA receptor hypofunction” in schizophrenia. Consistent with this prediction, the clinical trial provided proof-of-concept evidence that a novel compound with agonist activity at both mGluR2 and mGluR3 receptors had antipsychotic efficacy in schizophrenia (2).

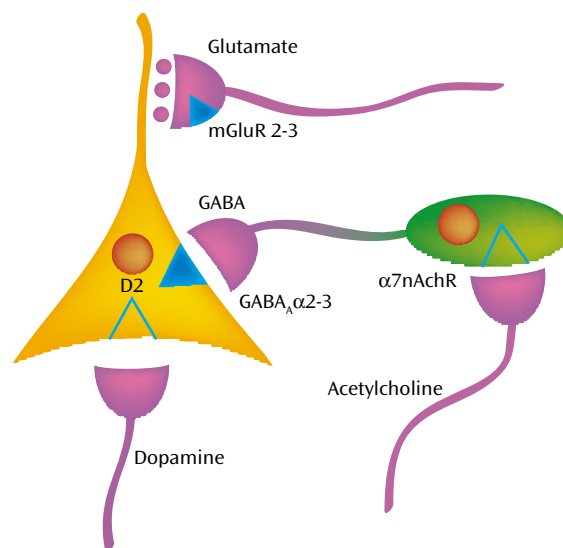
Although these initial findings are quite exciting, they raise a number of questions that are important for the continued development of novel antipsychotic medications with this mechanism of action. For example, what is the status of the targeted receptors in schizophrenia? Which regions of the brain contain altered levels of the receptors? Does the nature of the alterations in mGluR2 and mGluR3 receptors in schizophrenia suggest that the apparent antipsychotic efficacy of mGluR2/mGluR3 agonists is principally at one of these receptor subtypes? Until recently, studies were carried out with orthosteric agonists, which activate both receptor subtypes (mGluR2 and mGluR3) with equal affinity. However, mGluR2 and mGluR3 are desensitized differently (10), which raises the possibility of different roles for these two subtypes of group II mGluRs in the treatment development of psychiatric disorders.

In order to address these types of questions, Ghose and colleagues in this issue of the *Journal* (7) measure the protein levels of mGluR2 and mGluR3 receptors in postmortem

---

*“What is the status of the targeted receptors in schizophrenia?”*

---

FIGURE 1. Neuronal Hyperactivity as a Pathophysiological Feature of Schizophrenia<sup>a</sup>

<sup>a</sup> The diagram illustrates diminished GABAergic input from inhibitory interneurons (right) to cortical pyramidal neurons (left), which is one possible cause of neuronal hyperactivity in schizophrenia. Interneuron activity can also be diminished by the psychotomimetic drug phencyclidine, which blocks NMDA-type glutamate receptors that normally activate interneurons. Currently prescribed antipsychotic drugs block the D2 dopamine receptors that normally enhance the responsivity of cortical pyramidal neurons. New approaches under development include drugs that activate metabotropic glutamate receptors that decrease release of the excitatory neurotransmitter glutamate (7), drugs that directly activate GABA receptors (8), and drugs that activate interneurons through nicotinic receptors (9).

tissue samples of three cortical regions in subjects with schizophrenia and matched normal comparison subjects. The investigators also measure protein levels of the enzyme glutamate carboxypeptidase II (GCP II), which metabolizes *N*-acetylaspartylglutamate (NAAG), an endogenous agonist of mGluR3 (11). Relative to comparison subjects, individuals with schizophrenia had significantly higher levels of GCP II protein and lower levels of mGluR3 protein (with no difference in mGluR2 protein levels) in the dorsolateral prefrontal cortex. In contrast, no differences between subject groups were found for any of the three proteins in the temporal or motor cortex. The authors conclude that these findings implicate the mGluR3 receptor as the principal mediator of the antipsychotic action of mGluR2/mGluR3 agonists.

One approach to interpreting the meaning of these types of protein alterations involves determining how each alteration fits into the cascade of molecular features that characterize the underlying disease process of schizophrenia. That is, any given finding in a postmortem study could represent a cause (an upstream factor related to the pathogenesis of the illness), a consequence (a deleterious effect of a cause), a compensation (a response to either a cause or consequence that helps restore homeostasis in the brain), or a confound (a product of factors frequently associated with, but not a part of, the disease process or an artifact of the approach used to obtain the measure of interest) (12). Ghose and colleagues have carefully examined potential confounds in their study, and the available evidence excludes the usual suspects of between-group differences in subject characteristics (e.g., age and sex) or tissue quality (e.g., delay between time of death and tissue preservation) or of the effect of chronic exposure to antipsychotic medications. For example, the authors show that levels of mGluR3 and GCP II proteins, which have similar distributions in human and rodent brain, were not altered in rats exposed for 6 months to antipsychotic drugs.

As for any empirical data, the strongest argument that a given finding reflects the disease process is the independent replication of the findings using complementary research strategies that tend to be subject to a different set of confounds (e.g., postmortem studies

of GCP II and mGluR3 mRNA levels or in vivo measures of mGluR3 binding). A recent report by Gonzalez-Maeso and colleagues (13) demonstrated changes in mRNA expression of mGluR2-5-hydroxytryptamine 2A receptor (2AR) in psychosis, not mGluR3. In the post-mortem human brain, mGluR2 forms a protein-protein functional complex between serotonin and glutamate receptors (2AR-mGluR2). Thus, we cannot exclude the possibility that both receptors are involved in the development or potential treatment of schizophrenia.

An interesting question is regarding the potential relationship between the reciprocal alterations in GCP II and mGluR3 protein levels. Do they represent cause and consequence or consequence and compensation? For example, if the lower levels of mGluR3 protein reflect the effect of risk variants in the *GRM3* gene (14), then one might predict a compensatory response of lower levels of GCP II in order to increase levels of the endogenous mGluR3 agonist, NAAG, and thus augment stimulation of the apparent deficit in the number of mGluR3 receptors. Contrary to this prediction, the findings of Ghose and colleagues raise the possibility of two separate processes resulting in deficiencies in both an endogenous agonist for the mGluR3 receptor and the receptor itself that converge to produce a marked impairment in this signaling pathway in the dorsolateral prefrontal cortex of individuals with schizophrenia. The combined deficit could thus be considered to support the authors' interpretation that augmenting activity at the mGluR3 receptor accounts for the reported antipsychotic efficacy action of a mGluR2/mGluR3 agonist in schizophrenia (2). Evidence that the combined deficit is present in brain regions typically associated with psychosis (e.g., the striatum and superior temporal gyrus) would strengthen this interpretation. The presence of these alterations selectively in the dorsolateral prefrontal cortex raises the possibility that mGluR3 agonists might improve cognition in schizophrenia.

On the other hand, the combination of less endogenous ligands and fewer receptors raises the possibility that down-regulating mGluR3 signaling is a compensatory response to some other deleterious effect on brain function in the illness. Although the present findings do not immediately suggest a cause and compensation relationship, this interpretation might be consistent with findings in mice indicating that activation of mGluR2, but not mGluR3, receptors explains the reported antipsychotic efficacy of an mGluR2/mGluR3 agonist (15). Of course, multiple other proteins are also involved in regulating glutamate neurotransmission in the brain. A fuller knowledge of how the profiles of these proteins are altered across individuals with schizophrenia and across brain regions within individuals might both inform the relationships between such alterations and strengthen predictions regarding which molecules are most likely to be promising targets for novel pharmacological interventions.

## References

1. Moghaddam B, Adams BW: Reversal of phencyclidine effects by a group II metabotropic glutamate receptor agonist in rats. *Science* 1998; 281:1349–1352
2. Patil ST, Zhang L, Martenyi F, Lowe SL, Jackson KA, Andreev BV, Avedisova AS, Bardenstein LM, Gurovich IY, Morozova MA, Mosolov SN, Neznakov NG, Reznik AM, Smulevich AB, Tochilov VA, Johnson BG, Monn JA, Schoepp DD: Activation of mGlu2/3 receptors as a new approach to treat schizophrenia: a randomized phase 2 clinical trial. *Nat Med* 2007; 13:1102–1107
3. Javitt DC: Glutamate and schizophrenia: phencyclidine, N-methyl-D-aspartate receptors, and dopamine-glutamate interactions. *Int Rev Neurobiol* 2007; 78:69–108
4. Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, Heninger GR, Bowers MB Jr, Charney DS: Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans: psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Arch Gen Psychiatry* 1994; 51:199–214
5. Moghaddam B: Bringing order to the glutamate chaos in schizophrenia. *Neuron* 2003; 40:881–884
6. Moghaddam B: Targeting metabotropic glutamate receptors for treatment of the cognitive symptoms of schizophrenia. *Psychopharmacology (Berl)* 2004; 174:39–44
7. Ghose S, Gleason KA, Potts BW, Lewis-Amecua K, Tamminga CA: Differential expression of metabotropic glutamate receptors 2 and 3 in schizophrenia: a mechanism for antipsychotic drug action? *Am J Psychiatry* 2009; 166:812–820
8. Lewis DA, Cho RY, Carter CS, Eklund K, Forster S, Kelly MA, Montrose D: Subunit-selective modulation of GABA type A receptor neurotransmission and cognition in schizophrenia. *Am J Psychiatry* 2008; 165:1585–1593

9. Freedman R, Olincy A, Buchanan RW, Harris JG, Gold JM, Johnson L, Allensworth D, Guzman-Bonilla A, Clement B, Ball MP, Kutnick J, Pender V, Martin LF, Stevens KE, Wagner BD, Zerbe GO, Soti F, Kem WR: Initial phase 2 trial of a nicotinic agonist in schizophrenia. *Am J Psychiatry* 2008; 165:1040–1047
10. Iacovelli L, Molinaro G, Battaglia G, Motolese M, Di Menna L, Alfiero M, Blahos J, Matrisciano F, Corsi M, Corti C, Bruno V, De Blasi A, Nicoletti F: Regulation of group II metabotropic glutamate receptors by G protein-coupled receptor kinases: mGlu2 receptors are resistant to homologous desensitization. *Mol Pharmacol* 2009; 75:991–1003
11. Wroblewska B, Wroblewski JT, Pshenichkin S, Surin A, Sullivan SE, Neale JH: N-acetylaspartylglutamate selectively activates mGluR3 receptors in transfected cells. *J Neurochem* 1997; 69:174–181
12. Lewis DA, Gonzalez-Burgos G: Neuroplasticity of neocortical circuits in schizophrenia. *Neuropsychopharmacology* 2008; 33:141–165
13. Gonzalez-Maeso J, Ang RL, Yuen T, Chan P, Weisstaub NV, Lopez-Gimenez JF, Zhou M, Okawa Y, Callado LF, Milligan G, Gingrich JA, Filizola M, Meana JJ, Sealfon SC: Identification of a serotonin/glutamate receptor complex implicated in psychosis. *Nature* 2008; 452:93–97
14. Egan MF, Straub RE, Goldberg TE, Yakub I, Callicott JH, Hariri AR, Mattay VS, Bertolino A, Hyde TM, Shannon-Weickert C, Akil M, Crook J, Vakkalanka RK, Balkissoon R, Gibbs RA, Kleinman JE, Weinberger DR: Variation in GRM3 affects cognition, prefrontal glutamate, and risk for schizophrenia. *Proc Natl Acad Sci U S A* 2004; 101:12604–12609
15. Woolley ML, Pemberton DJ, Bate S, Corti C, Jones DN: The mGlu2 but not the mGlu3 receptor mediates the actions of the mGluR2/3 agonist, LY379268, in mouse models predictive of antipsychotic activity. *Psychopharmacology (Berl)* 2008; 196:431–440

**BARBARA WROBLEWSKA, Ph.D.**  
**DAVID A. LEWIS, M.D.**

*Address correspondence and reprint requests to Dr. Wroblewska, Department of Biology, Molecular Neurobiology Laboratory, Georgetown University, 37th and O St. NW, Washington, DC 20057; wroblewb@georgetown.edu (e-mail). Editorial accepted for publication April 2009 (doi: 10.1176/appi.ajp.2009.09040522).*

*Dr. Lewis currently receives investigator-initiated research support from the Bristol-Myers Squibb Foundation, Bristol-Myers Squibb Company, Curridium Ltd., Pfizer, and U.S. Pharmaceuticals Group; he has also served as a consultant in the areas of target identification and validation and new compound development for AstraZeneca Pharmaceuticals, Bristol-Myers Squibb, Hoffman-Roche, Eli Lilly, Merck, and Neurogen. Dr. Wroblewska reports no competing interests. Dr. Freedman has reviewed this editorial and found no evidence of influence from these relationships.*