



Trip Report for

**“2008 Annual Experimental Biology Conference,”
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Abstract: The 2008 Experimental Biology Conference was sponsored by The American Association of Anatomists (AAA); The American Association of Immunologists (AAI); The American Physiological Society (APS); American Society for Biochemistry and Molecular Biology (ASBMB); American Society for Investigative Pathology (ASIP); American Society for Nutrition (ASN); American Society for Pharmacology and Experimental Therapeutics (ASPET). This national conference was well attended by approximately 14,000 members from both academia and industry. The conference featured seminar presentations, poster sessions, exhibitions, and workshops in the areas of anatomy and physiology, biochemistry and molecular biology, immunology, nutrition, pathology, and pharmacology. This report highlights selected seminars presented.

“Treatments for Schizophrenia: Targeting Psychosis and Cognition”

Carol A. Tamming, (University of Texas Southwestern Medical Center), USA

Schizophrenia is a devastating disease that affects approximately 1% of the world’s population. The disease affects over 2.2 million people in the U.S. alone, which is approximately twice the number of people stricken with Alzheimer’s disease and virtually six times more than those diagnosed with insulin-dependent diabetes. The high level of prevalence enjoyed by this disease has ranked it among the top ten causes of disability worldwide. The current market for schizophrenia exceeds \$12 billion worldwide.

The symptoms of schizophrenia generally appear in late adolescence/early adulthood and can be compartmentalized into three specific categories. These include positive symptoms (auditory and visual hallucinations, disorganized speech, delusions), negative symptoms (social withdrawal, anhedonia, blunted affect, catatonia), and cognitive disorders (diminished capacity for learning and memory, attention and vigilance, and social cognition). Due to these debilitating symptoms, suicidality is common. While the exact causes of the disease are unknown, evidence suggests that it is a highly complex pathology involving a combination of genetic and environmental factors.

Current antipsychotics used to treat schizophrenia are divided into two classes: typicals and atypicals. Both classes are effective at diminishing the positive symptoms of the disease, but are marginally efficacious with regard to the negative and cognitive disorders. In addition, these drugs have a severe side effect profile that includes extrapyramidal side effects, QTc prolongation, severe weight gain, and associated metabolic disorders. This side effect liability coupled with the lack of overall efficacy creates a severe patient compliance problem. Therefore, more efficacious and safer antipsychotics are drastically needed.

The activity of the typicals and atypicals is attributed primarily to their dopaminergic (D2) antagonist activity. These drugs are potent at this receptor but also hit many other off-target receptors. The discovery of the typical/atypical mode of action via dopaminergic activity gave rise to the dopamine hypothesis for schizophrenia. This

hypothesis states that the underlying pathology of the disease can be attributed to hyperdopaminergic activity in the brain. This hypothesis accounts for amelioration of positive symptoms with dopamine antagonist administration and production of hallucinations when agonists are administered. However, the hypothesis does not account for the negative and cognitive symptoms.

The dopamine hypothesis is ultimately being supplanted by the glutamate hypothesis in light of the discovery that NMDA antagonists elicit all of the symptoms of schizophrenia in healthy individuals. Glutamate is the principle excitatory neurotransmitter in the CNS and its activity is modulated by two types of receptors; ionotropic (NMDA, AMPA, kainate) and metabotropic (mGluRs). The NMDA (N-methyl-D-aspartic acid) receptor is involved in fast excitatory neurotransmission and plays critical roles in several CNS processes, including long term potentiation (cognition, learning, and memory). The discovery that NMDA hypofunction may be playing a critical role in the underlying pathology of the disease has caused a paradigm shift in the search for newer and better antipsychotics. The search for new antipsychotics is no longer exclusive for monoaminergic targets, but includes other targets on other circuits that may also be highly relevant to the pathology of the disease. Dr. Tamminga highlighted several approaches that include classical monoaminergic targets as well as new targets. The current approaches are also placing a greater emphasis on cognition, which is markedly impaired in most schizophrenics and prevents them from being functioning members of society. The targets described are listed below:

- 1) Nicotinic-acetylcholine ($\alpha7, \alpha4, \beta2$) receptor partial agonists (cognition, cholinergic pathway).
- 2) Selective D1 and 5-HT_{2A} antagonists.
- 3) Selective 5-HT₆ antagonists (cognition).
- 4) Muscarinic (M1, M4) acetylcholine agonists and positive allosteric modulators (cholinergic and glutamatergic pathways).
- 5) Ampakines (glutamatergic pathway).
- 6) Glycine transporter-1 inhibitors (glutamatergic pathway).
- 7) mGluR_{2/3} agonists and positive allosteric modulators (glutamatergic pathway).
- 8) H₃ antagonists/inverse agonists (cognition, histaminergic pathway).
- 9) GABA_A agonists (GABAergic pathway).

The targets listed above are being investigated as a means by which to ameliorate all of the symptoms associated with schizophrenia. However, it is believed that potential drugs emerging from these targets will not be stand alones but adjuncts. In addition, Dr. Tamminga believes that improvement in cognition will not be achieved by polypharmacy alone, but via a combination of pharmacotherapy and cognitive remediation. The discovery of the potential role of NMDA hypofunction in schizophrenia has opened the doors to other previously not considered targets that may be highly relevant in the pursuit of an efficacious and safe antipsychotic.

“Allosteric Activators of Muscurinic Receptors as a Novel Approach for the Treatment of Schizophrenia”

P. Jeffery Conn, (Vanderbilt University Medical Center), USA.

The NMDA receptor is a glutamatergic ion channel involved in fast excitatory neurotransmission. It plays key roles in a variety of functions in the CNS, most notably long term potentiation and neuronal plasticity. The discovery that the NMDA antagonists PCP and ketamine elicit the symptoms of schizophrenia in healthy individuals has led to the NMDA hypofunction hypothesis for the disease. This hypothesis states that hypofunction of the NMDA receptor leads to hyperactivity of non-NMDA glutamatergic and dopaminergic/serotonergic systems in the limbic and cortical areas of the brain. It is believed that NMDA hypoactivity may be at the root of the underlying pathology of schizophrenia.

The NMDA receptor is both a ligand and voltage gated ion channel comprised of two different subunits (NR1 and NR2). Modulation of NMDA activity is carefully orchestrated by multiple systems and other receptors. Embedded within the central pore of the channel is a magnesium ion, which holds it in the closed position. This ion is extruded by depolarization of the cell. Once the channel is cleared, both glutamate (endogenous agonist) and glycine (requisite co-agonist) must bind before calcium and sodium can flow into the cell and potassium out. Modulation of the receptor is also achieved with polyamines, which bind to a specific site.

There are specific receptors that potentiate NMDA by causing a depolarization of the cell that ultimately leads to magnesium ion extrusion. These receptors include mGluR5, AMPA, and the muscurinic-acetylcholine receptors (mAChR) M1 and M4.

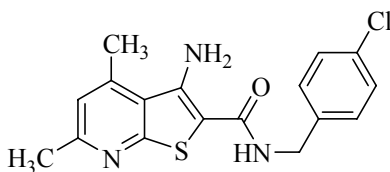
mAChRs have been shown to regulate NMDA currents in the forebrain. The M1, M4 agonist xanomeline has been shown to induce robust improvements in behavioral disturbances in Alzheimer's disease patients and has shown efficacy for the positive and negative symptoms of schizophrenia in a double-blinded, placebo controlled study. However, xanomeline suffers from side effects due to activity at several off-targets (including the other mAChRs) and is susceptible to agonist liabilities (liabilities include adverse side effects associated with excessive receptor activation, desensitization due to receptor downregulation and/or internalization, and degradation of neuronal activity dependence of receptor activation due to disruption of the natural release of the endogenous neurotransmitter).

Dr. Conn presented research focused on the discovery and pharmacology of novel M1 and M4 positive allosteric modulators (PAMs) and allosteric agonists. PAMs bind to an allosteric site that has had less evolutionary pressure for conservation relative to the orthosteric site. As such, structurally diverse ligands have been found to bind to specific receptors at these sites with a high level of selectivity. In addition, PAMs are inherently devoid of activity on their own. They simply potentiate the receptor toward the

endogenous ligand. Thus, PAMs may provide a means by which one can increase stimulation at a specific receptor selectively and without the liabilities associated with direct agonists. The high level of selectivity could also provide a better safety/side effect profile.

Dr. Conn has demonstrated that the selective M4 PAM VU10010 (Figure 1) potentiates the receptor nearly 50-fold (leftward shift in EC_{50}) in acetylcholine concentration response curves (GTP γ^{35} S binding assay).

Figure 1.

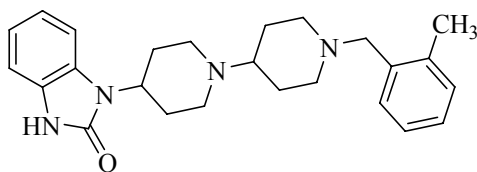


VU10010

The compound is 100 nM for M4 with no appreciable binding at the other mAChRs or any other family A GPCRs. The compound was found to bind at an allosteric site, not the orthosteric acetylcholine site. This compound has also been shown to reverse amphetamine-induced hyperlocomotion in rodents, an animal model predictive of potential antipsychotic activity.

Dr. Conn has also discovered the novel allosteric agonist TBPB via a high throughput screening (HTS) campaign (Figure 2).

Figure 2.



TBPB

Unlike PAMs, allosteric agonists are active; yet do not bind to the highly conserved orthosteric site. TBPB was found to be highly selective for M1 and has shown efficacy in the following animal models predictive for potential antipsychotic activity:

- 1) TBPB reduces amphetamine-induced hyperlocomotion.
- 2) Reverses amphetamine-induced disruption of PPI.

- 3) Induces changes in cFos expression similar to the atypicals.
- 4) Does not induce multiple, peripheral adverse effects mediated by M2 and M3.
- 5) Does not occupy D2 receptors.

The M1 allosteric agonist TBPB and the M4 PAM VU10010 hold promise as potential antipsychotics that are devoid of monoaminergic activity. Such antipsychotics could lead to safer and more efficacious treatments for schizophrenia.

“AMPA Receptor Potentiation: A Core Antidepressant Pathway?”

J.M. Witkin, (Eli Lilly & Co.), USA.

Depression is one of the most prevalent of all mental illnesses. It is estimated that nearly 20% of the adult population in the U.S. will suffer from the disorder at some point in life. All of the current antidepressants prescribed today (tricyclics, MAOIs, SSRIs, and SNRIs) work by increasing monoamine concentrations in the synapse. The biogenic amine approach for treating depression suffers various shortcomings. These include a slow onset of action (typically three weeks), low efficacy across the patient population (30% to 40% do not respond to treatment), high remission rates, and various side effects.

The therapeutic lag associated with antidepressants has led investigators to take a closer look at the adaptive changes occurring in the brain downstream from the monoaminergic synapses that are required for a therapeutic response. Most notably, research is focused on pathways that enhance production of brain derived neurotrophic factor (BDNF) which leads to increased neuronal viability and generation in specific regions of the brain.

Brain-derived neurotrophic factor (BDNF) is a member of the nerve growth family of neurotrophins found in the brain and the periphery. It is involved in neural protection and neural generation and can modulate the function, survival, sprouting, proliferation, maintenance, and recovery of neurons. Data suggests that increased levels of BDNF by antidepressants are responsible for efficacy in symptom relief. Decreased levels of BDNF have been reported in patients with severe depression as well as decreased hippocampal volume. Researchers have found that antidepressant treatment and electroconvulsive shock increases BDNF levels and that exogenous infusion of BDNF increases neurogenesis and produces antidepressant-like activity in animal models. Recent preclinical findings suggest a strong linkage between antidepressant efficacy and neurogenesis. Therefore, receptors that are closely linked to enhancing BDNF production may provide antidepressant effects with a much faster onset of action and with fewer side effects. One such receptor that may provide a novel, non-monoaminergic antidepressant is the glutamate AMPA receptor.

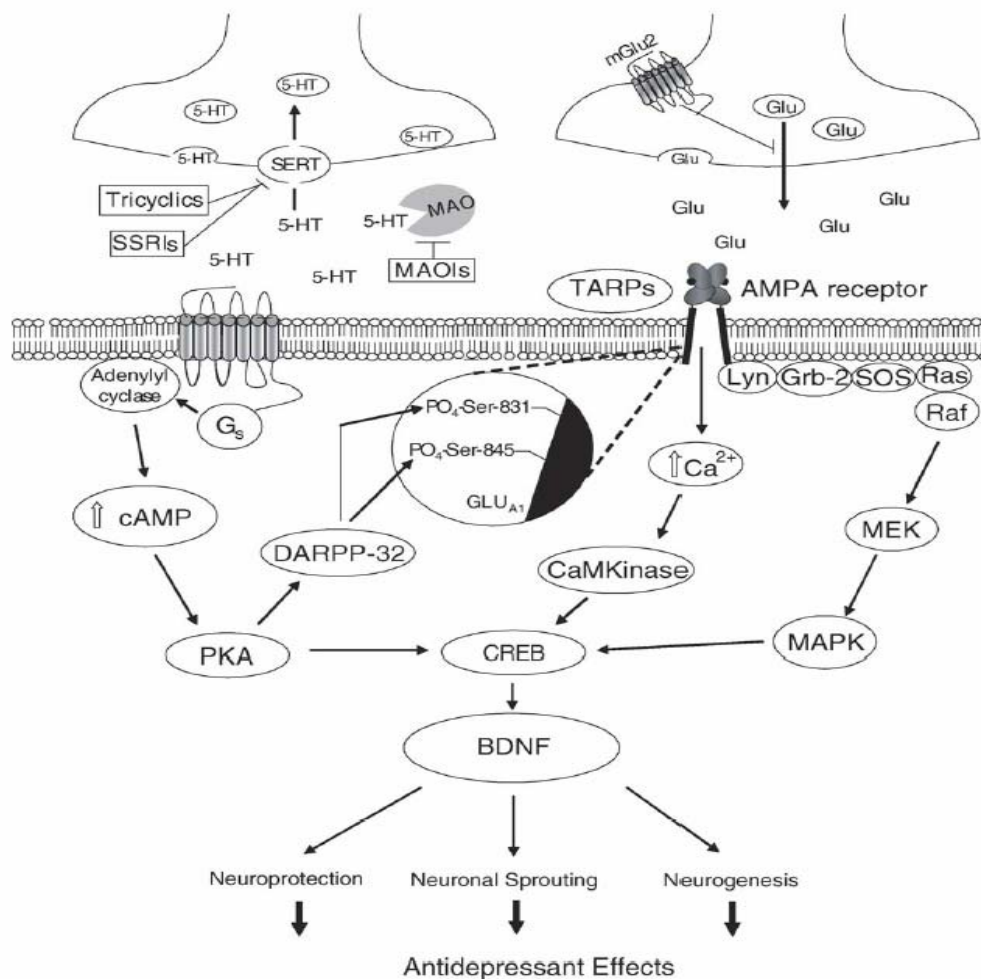
Glutamate is the principle excitatory neurotransmitter in the CNS and its activity is modulated by two types of receptors; ionotropic (NMDA, AMPA, kainate) and metabotropic (mGluRs). The AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor family includes four different genes termed GLUA1-4 that contain a large extracellular NH₂-terminal domain (“clamshell”) and four

hydrophobic domains labeled M1-M4. AMPA mediates fast excitatory synaptic transmission and evidence suggests that functional receptors are tetramers that are composed of one or more subunits (GluR1-4), yielding either homomeric or heteromeric configurations.

There is an emerging body of evidence that suggests potentiation of the AMPA may play a key role in current biogenic amine antidepressant efficacy. It has been found in animal models that chronic treatment of desipramine and paroxetine led to upregulation of the AMPA subunits GluR1 and GluR2/3 in rodent hippocampal extracts. It has also been shown that chronic administration of fluoxetine to rodents led to increased phosphorylation of the GluR1 subunit (via DARPP-32), which potentiates AMPA activity.

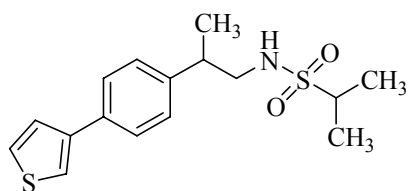
The current theory behind conventional biogenic amine antidepressant activity focuses on the cellular cascades that lead to an increase in cAMP production. This leads to a downstream increase in BDNF production (via PKA and CREB) and ultimately an antidepressant response. It is believed that AMPA activity can lead to an increase in BDNF production by means of more direct and more than one cellular pathway (Figure 3). As an ion channel, AMPA receptors increase calcium ion influx, which has a direct effect on BDNF production. AMPA has also been shown to increase BDNF production via the MAP kinase pathway.

Figure 3.



Dr. Witkin presented data for the AMPA positive allosteric modulator LY392098 (Figure 4).

Figure 4.

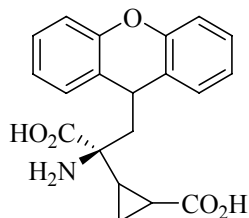


LY392098

LY392098 was efficacious in the forced swim and tail suspension animal models (mouse and rat), which are predictive of potential antidepressant activity. The compound also led to an increase in BDNF mRNA production in the rat hippocampus. LY392098 was also efficacious in the novel object recognition model, demonstrating precognitive effects of AMPA receptor potentiation.

Dr. Witkin also provided data for the mGluR2 antagonist LY34149 (Figure 5).

Figure 5.



LY34149

mGluR2 is a member of the family C GPCRs and is a group II mGluR. Group II (mGluR2, 3) and III (mGluR 4, 6-8) are negatively coupled to adenylyl cyclase, thus leading to a decrease in neurotransmission upon activation. mGluR2 functions primarily as an autoreceptor located presynaptically and is involved in the feedback control of glutamate release and in the regulation of other neurotransmitters. Inhibition of this receptor leads to an increase in glutamate concentration in the synapse. This in turn could enhance AMPA activity, which could ultimately lead to antidepressant effects. In fact, LY34149 displayed efficacy in the mouse forced swim test and also led to enhanced cortical acetylcholine efflux, which gave rise to wake promoting and cognitive activating effects. The preclinical *in vivo* antidepressant activity observed for both AMPA PAMs and allosteric agonists and mGluR2 antagonists demonstrates that glutamatergic systems are highly relevant in depression. Further investigation into the properties of these compounds could lead to faster acting and more efficacious antidepressants.