Antipsychotic Drug Mechanisms & Schizophrenia
Schizophrenia: characteristics

- Confused Thinking
- Hallucinations
- False beliefs
- Paranoia
- Cognitive dysfunctions
  - Attention & memory
- Decreased social engagement
- Lack of motivation
- Anxiety
- Depression
- Irritable Bowel Syndrome

Typically begins in early 20's.
Schizophrenia: Positive & Negative Symptoms

**Positive Symptoms**
*Feelings/behaviors usually not present*
- Hallucinations
- Delusions
- Disorganized speech

**Negative Symptoms**
*Lack of feelings/behaviors that are usually present*
- Apathy
- Depression
- Anxiety
- Social Withdraw
### Schizophrenia: how does it happen?

#### Environment

<table>
<thead>
<tr>
<th>Factor</th>
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<tbody>
<tr>
<td>Cannabis &amp; other substance abuse</td>
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<tr>
<td>Infection (fetal development)</td>
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<tr>
<td>Parental Age</td>
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<tr>
<td>Poor neo-natal nutrition &amp; stress</td>
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<tr>
<td>Intestinal Flora &amp; Intestinal tract dysfunction</td>
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#### Genetics

<table>
<thead>
<tr>
<th>Gene/Protein</th>
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<tbody>
<tr>
<td>COMT: DA</td>
</tr>
<tr>
<td>DISC1: doesn’t tell us much</td>
</tr>
<tr>
<td>NOTCH4: neuroendocrine</td>
</tr>
<tr>
<td>Histone protein loci</td>
</tr>
<tr>
<td>Zinc finger protein 804A</td>
</tr>
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Schizophrenia: the brain

- Frontal Lobe
- Reduced Brain Volume
- Hippocampus
- Temporal Lobe

*Reported: even more atrophy than Alzheimer's
Schizophrenia: mechanisms, traditional views

The Dopamine Hypothesis

- Hyperdopaminergic activity
- Amphetamines make worse & phenothiazine makes better
- In particular, excessive D2 (the inhibitory type of DA)
Schizophrenia: mechanisms, traditional views

The Serotonin idea

- Serotonin Abnormalities, too much in blood & too little in CSF
- LSD & other hallucinogens have a similar structure
- Risperidone & clozapine: 5-HT antagonists, reduce negative symptoms
Schizophrenia: mechanisms, traditional views

The Glutamate idea

- Reduced function of NMDA glutamate receptors
- Lack of glutamate receptors, mGlu2/3
- Glutamate blocking drugs like ketamine & phencyclidine mimic Schizophrenia

*Problem:* Positive & Negative Symptoms affected by neurotransmitters differently. Hallucinations & delusions probably go down different pathways than the cognitive impairments & depression.

Can one type of antipsychotic solve all?
Schizophrenia: Mechanism, new views

Antipsychotic drug mechanisms: links between therapeutic effects, metabolic side effects and the insulin signaling pathway

RR Girgis¹,², JA Javitch¹,² and JA Lieberman¹,²
FGA’s Vs. SGA’s

*First generation antipsychotics versus second generation antipsychotics*

- First generation: mainly work through blocking D2 receptors
  - Causes EPS’s: Extrapyramidal Symptoms, as seen in Parkinson’s due to the lack of DA

- Second generation: they also block D2 receptors, but not nearly as much.
  - Also inhibit 5HT1A serotonin receptors (inhibit cortical pyramidal neurons)
  - Hit and run?
  - They are more effective for the psychosis
  - Don’t cause as harsh of EPS’s
  - BUT... they cause a lot of metabolic disturbances
  - The exact mechanism is unknown, but the endocrine issues point to the insulin pathway
SLA's

- Hyperglycemia
- Hyperlipidemia
- Insulin resistance
- Obesity
- Diabetes
**SGA's**:
- Clozapine
- Olanzapine

**EPS**s
- Few EPS's
- ↑ Metabolic Issues

**D₂ Receptor Block**

**FGA's**:
- Chlorpromazine
- Haloperidol

↑ EPS & Few Metabolic Issues
Via D₂ Receptor Block

Glu Transporter?
Akt?
GSK?
Hypothesis...

There are abnormalities in the insulin signaling pathway of the brain’s of those with schizophrenia.

Maybe this is a principle of what is going on in the brain of schizophrenics and perhaps this is why the SGA works so well, because it targets the insulin pathway, resulting in a by-product of metabolic malfunctions.

Relationship between effectiveness of antipsychotic and metabolic disturbances.
GSK
- Mood stabilization
- Neuroplasticity
- Microtubule dynamics
- Neurogenesis
- Gene expression

GLU
DA
5-HT

Akt
- Regulation of cell cycle
- Apoptosis
- DNA synthesis

* you want to be in homeostasis
Rat Study- Inducing Schizophrenia

NMDA (GLU) & D₂
PCP: NMDA antagonism: Inhibiting excitation
D₂↑: increase inhibition
SER↓: decrease synaptic connections

↑DA & ↓NMDA = ↓phosphorylation of Akt & GSK

↓Akt & ↑GSK
Akt
Abnormalities of the insulin signaling pathway

The insulin signaling pathway in individuals with schizophrenia and the effects of antipsychotic drugs is complicated...

- Human studies conducted on people exposed to FGAs and/or SGAs
- Presence or absence of the disease complicates the comparisons
- Comparisons are required between human and nonhuman data and between studies involving different cell types
- Issues about specimen processing add more complexity to interpretation of the data (too small sample size)

Despite these limitations...it is important to review the findings
Genetic Alterations

- Genetic alterations have been noted in individuals with schizophrenia within Chinese and African American populations.
- Seems to be a relationship between components of phosphoinositide signaling (PIP5-K2A) and schizophrenia have been observed:
  - Phosphoinositide signaling: cellular phospholipids, serve as regulatory molecules, and help to modulate lipid distribution and metabolism via the relationship with lipid transfer proteins.
- Although...one study of IGF-1 in a Caucasian population found no association with schizophrenia.
Neuropathological Data

Little neuropathologic data that suggest there are abnormalities of the proximal components of the insulin signaling pathways

- STUDY: Individuals with schizophrenia who were all treated with FGAs, except one on clozapine (SGA)... showed decreased density of PKC in parahippocampal region when compared to controls (dead males with no schizophrenia) with no comparison between PKC and antipsychotic dose
  - asymmetry of this region is seen in schizophrenia
Neuropathological Data

- Similar analysis showed no difference when looking at the striatum (critical for reward system) and in the platelets of people with schizophrenia when compared to control group
Neuropathological Data

Haloperidol (FGA) exposure in rats showed increased PKC activity in the frontal cortex...BUT in another study, rats treated for 4 weeks with Haloperidol (FGA) or Clozapine (SGA) had NO CHANGE in PKC levels in the frontal cortex, basal ganglia, or the olfactory tubercle.
Neuropathological Data

All these data indicate LITTLE correlation between antipsychotic drugs and components of insulin signalling pathways...which give support to the observations that the interactions between the D2 receptor/antipsychotic drugs and insulin signaling pathways at the level of Akt

- STUDY: suggest that PKC is involved in increasing the effects of clozapine (SGA) on NMDA signaling (synaptic plasticity and memory function) BUT no connection was really made about the interaction with the insulin signaling pathway
Other Neuropathological Data

Other data are consistent with insulin resistance and hyperinsulinemia after treatment with antipsychotic drugs

- Olanzapine (SGA) has been shown to inhibit insulin stimulated activation of PI3K in rat skeletal muscle cell line (cells within a uniform genetic makeup) AND upregulates insulin-2 in rat frontal cortex
- Zhao et. al. examined the insulin receptors in the dorsolateral prefrontal cortex of individuals receiving FGAs and SGAs...found that both the total and activated insulin receptors were REDUCED in the individuals
Akt and Schizophrenia...Genetic Alterations?

- Genetic abnormalities of Akt were found in individuals with schizophrenia and was supported by finding an Akt1 haplotype (group of genes within an organism that was inherited from a single parent) associated with lower levels of Akt1...related to schizophrenia in Northern European populations
- There was a positive correlation between Akt1 and schizophrenia in Caucasian, Iranian, Japanese and Chinese populations...but not in two Japanese populations, a Taiwanese population, and a Caucasian population
Akt and Schizophrenia...

- Potential confound: phosphorylation levels of Akt is closely related to the peri-mortem condition of tissue (e.g., temperature, pH, etc).

- However... data shows that decreased levels of total and phosphorylated Akt in schizophrenia...this is consistent with theories of schizophrenia and overactive dopaminergic systems:
  - mesolimbic areas of the brain or NMDA receptor hypofunction
Examined dorsolateral prefrontal cortex of individuals with schizophrenia, individuals receiving FGAs, and individuals receiving SGAs. Studey demonstrated decreased expression of Akt1 were found in dorsolateral prefrontal cortex in another sample, whereas other studies report no alterations in frontal cortex Akt or phosphorylated Akt in individuals with schizophrenia. Decreased levels of Ser473-Akt in individuals receiving FGAs and individuals receiving SGAs, demonstrated, whereas other studies report no alterations in frontal cortex Akt or phosphorylated Akt in individuals with schizophrenia.
More studies...

- Emamian et. al. saw decreased levels of total Akt in lymphocyte-derived cell line, frontal cortex and hippocampus of individuals with schizophrenia.

- Primary neuronal culture, inhibition of DISC1 (gene implicated in schizophrenia), led to decreased phosphorylation of Akt but stable levels of Akt overall.
  - The disruption of DISC1 and possible predisposition for psychiatric conditions needs to be further studied.
Akt and Antipsychotic Drugs

- Clozapine (SGA) increases phosphorylation of Akt in neuroblastoma cells
  - Rat study showed that 5 days of clozapine (SGA) and chlorpromazine (FGA) increased phospho-Ser473-Akt without increased total Akt levels
- Olanzapine (SGA): shown to increase levels of phospho-Ser473-Akt in PC12 cells (combination of neuroblastic and eosinophilic cells) although total Akt stayed the same
  - Effect was dependent on PI3K...suggests an additional mechanism of antipsychotic effects on Akt and may be related to differences in cell line
More Akt and Antipsychotic Drugs observations...

- Chlorpromazine (FGA), clozapine (SGA) and fluphenazine (FGA) did not lead to increased phosphorylation of Akt in PC12 cells.

- Fluphenazine (FGA), chlorpromazine (FGA) and haloperidol (FGA) decreased the nerve growth factor-induced phosphorylation of Akt at Ser473 in PC12 cells, which is consistent with another study that showed that acute haloperidol (FGA) treatment of rat neurons decreased levels of phospho-Ser473-Akt.
These results underscore and illuminate differences in efficacy between antipsychotic drugs (more effects on Akt of olanzapine (SGA) and clozapine (SGA) than other antipsychotics)

HOWEVER...limited by nearly cytotoxic (quality of being toxic) doses of antipsychotic drugs used in some of the experiments may contribute to discrepant effects on Akt
Antipsychotic drugs seem to increase phosphorylation of Akt...antagonistic of what occurs in schizophrenia (decreased Akt) and predictable based on dopamine and NMDA receptor hypofunction hypothesis
● Conflicting Studies
  ○ Genetic Relationship between GSK-3B and paranoid subtype in Italians and Chinese
  ○ Not in Italian, Finnish, Japanese, Chinese, and Korean Populations

● Examined GSK-3 isoforms
  ○ Isoform: Two functionally similar proteins with nonidentical amino acid sequence

● Generally Found:
  ○ Decreased levels of GSK-3
  ○ Increased activity of GSK-3
  ○ Consistent with decreased levels of Akt & predictions of DA & NMDA receptor hypofunction hypothesis

Phosphorylation
  • Addition of a Phosphoryl Group $(PO_3)^{2-}$
  • Turns protein enzymes on and off
No correlation b/w Antipsychotics & GSK-3β

No alterations of GSK-3β
No correlation w/ lifetime antipsychotic or lithium use

↓ GSK-3β mRNA
⊗ GSK-3α mRNA
**Lymphatic system**

**Lymphocyte**

**Phosphorylation levels of GSK-3β**
- @ Ser9 — decreased
- @ Tyr219 — normal

Ser9: the site where Akt is phosphorylated

Supported w/ studies:
- ▼ GSK-3β levels @ Pre-FC
- ▼ phospho-Ser9-GSK3β @ FC
- [they also?] ▼ GSK3β found @ CSF

◆ Still NO correlations w/:
  - Antipsychotic dose
Dorsolateral prefrontal cortex

Zhao et al.

Patients receiving FGA (or SGA)

\[ \uparrow GSK-3\alpha \]

\[ \downarrow GSK-3\beta \]

\[ \times \text{phosphorylated forms of enzymes} \]

\[ \uparrow \text{activity of enzyme} \]
Hippocampus damage is mostly associated with anterograde amnesia.

Evidence shows hippocampus abnormalities in schizophrenic patients.

Rats w/ hippocampal damage

↓ GSK-3β @ frontal C. (Only before puberty)

× total GSK-3 (α & β) (indep. of age)

• Rats given FGA/SGA showed NO change in

  × GSK-3β
  OR
  × GSK-3 (α & β)
Rat GSK-3β levels **not** affected by stress

- altered GSK-3β levels in Schizophrenia ≠ disease ≠ stress response
No differences in GSK-3β.
Previously shown:
- Levels GSK-3β
- Activity GSK-3

- GSK-3α or -β mRNA
- GSK-3β protein
- Total GSK-3(α & β)

Confounds

Hippocampus

↓ GSK-3β protein levels
GSK-3β
phosphorylated GSK-3β
GSK-3α δ - β mRNA, GSK-3β

Frontal cortex
lymphocytes

Previously Shown
↓ GSK-3β levels @ pre-FC
↓ phospho-Ser9-GSK3β @ FC
[they also] ↓ GSK3β @ CSF
<table>
<thead>
<tr>
<th>#</th>
<th>Drug</th>
<th>Effect</th>
<th>Brain Area</th>
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<tbody>
<tr>
<td>Sub-Chronic</td>
<td>Haloperidol</td>
<td>↑ GSK-3</td>
<td>Striatum</td>
</tr>
<tr>
<td>Sub-Chronic</td>
<td>Risperidone</td>
<td>↑ GSK-3</td>
<td>Pre Frontal Cortex</td>
</tr>
<tr>
<td>Sub-Chronic</td>
<td>Clozapine</td>
<td></td>
<td>Ventral Midbrain</td>
</tr>
<tr>
<td>Sub-Chronic</td>
<td>Hal. Ris.</td>
<td>↑ phospho-Ser9-Gsk-3β</td>
<td>PFC</td>
</tr>
<tr>
<td>Chronic (sub-acute)</td>
<td>Hal. Ris.</td>
<td>↑ GSK-3 (α &amp; β)</td>
<td>Pre Frontal Cortex</td>
</tr>
<tr>
<td>Chronic</td>
<td>Hal.</td>
<td>↑ phospho-Ser9-Gsk-3β</td>
<td>Ventral Midbrain</td>
</tr>
<tr>
<td>Chronic</td>
<td>Hal.</td>
<td>↓ phospho-Ser9-Gsk-3β</td>
<td>FC</td>
</tr>
<tr>
<td>Chronic</td>
<td>Cloz.</td>
<td>↑ phospho-Ser9-Gsk-3β</td>
<td>FC</td>
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Confounds?
1. rats vs mice
2. dif. Haloperidol doses (10 vs 1 mg kg⁻¹)
3. dif. lengths of exposure (21 vs 12 days)
4. anesthetic & post-mortem processing

Chronic treatment nec.?

* in mice (similar to Akt)

* in rats
<table>
<thead>
<tr>
<th>Drug</th>
<th>Affect</th>
<th>Where</th>
<th>Notes</th>
</tr>
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</table>
| Risperdine| \(\uparrow\) phospho-Ser21-\*Gsk-3\*
\(\times\) total Gsk-3\*
\(\times\) phospho-Ser-473-Akt
\(\times\) phospho-Thr308-Akt | cortex, striatum, hippocampus, cerebellum | Not haloperidol
\*MOUSE
\*Augmented w/ Imipramine/Fluoxetine
Not consistent |

**Affect**

\(\text{Clozapine} = \text{Olaprazine} > \text{Quetiapine} > \text{Ziprasidone}\)

\*Consistent w/ data:

\(\text{Clozapine} \& \text{Olaprazine} = \text{greatest metabolic disturbance} = \text{most effective SGA}\)
<table>
<thead>
<tr>
<th>Drug</th>
<th>Effect</th>
<th>Tissue/Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clozapine</td>
<td>↑ phospho-Ser4-Gsk-3β, neuroblastoma cells</td>
<td><em>mouse</em></td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>5 days, active, non-phospho-GSK-3β, neuroblastoma</td>
<td><em>mice</em></td>
</tr>
<tr>
<td>Clozapine</td>
<td>28 days, ↓ phospho-Ser-21-GSK-3β, total GSK-3β</td>
<td></td>
</tr>
<tr>
<td>Clozapine</td>
<td>Dephosphorylation of GSK-3β &amp; Ser9, neuroblastoma</td>
<td></td>
</tr>
<tr>
<td>Olanzapine</td>
<td>inhibits insulin-stim. phosphorylation (inactivates) GSK-5(α &amp; β), rat skeletal muscle cell line</td>
<td></td>
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</table>

**Neuroblastoma Cells** - A type of cancer that starts in certain very early forms of nerve cells found in embryo of fetus

**Glioblastoma** - Malignant tumor of the brain or spine

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**Discrepancies**

- but if true, = peripheral insulin resistance (side effect of olanzapine)
GSK

generally:

↑ phosphorylation and (↑ inactivation)

GSK

mood stabilization
neuroplasticity
microtubule dynamics
neurogenesis

gene expression

In schizophrenia:

D₂ receptor regulates

↑ D₂ =

Insulin/IGF1

PKC

PI3 kinase

PIP₃

AKT

GSK-3

eIF2B

More inhibition of glycogen synthase

Glycogen
Summary

1) Possible alterations of Akt, certain phosphoinositide-relating genes, and GSK-3B

2) There are inconsistencies in possible inherent abnormalities in the insulin signaling pathway

3) Preliminary evidence suggests that these effects may occur more (or to a greater degree) with SGAs
Functional Selectivity as a potential mechanism

- More research is needed
- Curious that antipsychotic drugs with the greatest efficacy also have the greatest metabolic effects
- Can recent studies be used as evidence that Dopamine-2 receptors are indeed linked to insulin pathway in a more unitary way?
Functional Selectivity as a potential mechanism

- One possibility: clozapine and olanzapine have different targets than other (previous) antipsychotic drugs, therefore Dopamine-2 receptor blockade is irrelevant
  - Unlikely, because no antipsychotic effect has been observed in agents without D2 receptors
  - Exception of mGlu2/3 receptor agonist
  - Maybe D2 is relevant to a lesser degree, but SGAs act on other targets, like histamine-1, serotonergic or muscarinic receptors
Functional Selectivity as a potential mechanism

- Another possibility: different drugs act differently on D2 receptors that lead to differences in signalling but we simply can’t visualize it just yet
  - Drugs can enhance signaling through insulin pathway (Akt/GSK)
  - Effects might be more qualitative rather than quantitative (*all or nothing effects*), meaning that maybe insulin pathway is closest to D2 receptors that we know of

- Functional selectivity - binding of different psychotropic agents to the same G-coupled-receptor and evoking different effects
  - Ligands might be able to induce different and selective conformational changes in their receptors
  - Ligands might activate pathways based on the existence of certain key signal transduction proteins (e.g. arrestins)
Drug Selectivity

**Receptor Selectivity**
Selectivity for Different Receptor Subtypes

**Functional Selectivity**
Selectivity for Different Signaling Pathways Coupled to the Same Receptor
Functional selectivity as a potential mechanism

- Opportunities (Problems)
  - Need to better characterize insulin’s importance in schizophrenia
  - By using cerebrospinal fluid and/or peripheral tissue insulin measurements before and after taking antipsychotic drugs could lead to better understanding the insulin pathway and schizophrenia
  - Or could use D2 cell culture to look for functional selectivity. In vitro studies.
Conclusion

- Clinical observation supports current hypothesis that insulin signaling pathway is linked to schizophrenia, based on the efficacy of a drug and the side effects that it induces - metabolic disorders, however direct causation is not understood
- “However, the data that associate antipsychotic action with the insulin signaling pathway remain preliminary and sometimes contradictory”
The Future of Antipsychotic Drugs

- **2009- Iloperidone**
  - **Pros**
    - Only 13% of users have serious weight gain
  - **Cons**
    - Major side effects with alcohol (the drug is metabolized in the liver)

- **2010- Eli Lilly’s LY2140023**
  - Metabrotropic glutamate receptor (mGluR) agonist
  - No metabolic side effects

- **2015- Vraylar**
  - For bipolar disorder and schizophrenia
  - Partial agonist of the D2 receptor and the 5-HT1A receptor, and full agonist of the 5-HT2A receptor
Conclusion

- No idea why there is insulin resistance in periphery
- Why is there therapeutic effects centrally and detrimental effects peripherally?
- Further research needed