Target based biomarker selection –

Mineralocorticoid receptor related biomarkers and treatment outcome in depression

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Alec Coppen, The biochemistry of Affective Disorders:

1. Monoaminehypothesis
2. Stress-Hormone-Hypothesis
3. Electrolyte-Hypothesis
Alec Coppen, 1969:

“In view of the considerable disturbances in electrolyte distribution found in depression studies on aldosterone secretion would be most valuable”
"Stress and Disease", Selye 1956:

**P-C:**
Prophlogistic corticoids/ Mineralocorticoids:
(aldosterone)

**A-C:**
Antiphlogistic corticoids/ glucocorticoids:
cortisol

Mineralocorticoids are recognized early as pro-inflammatory
Aldosterone:

\[ \Rightarrow \text{Na}^+\text{-reabsorption} \]
\[ \text{K}^+\text{-excretion} \]
\[ \text{Mg}^{2+}\text{-excretion} \]
Is there a role for MR/RAAS in major depression?
Hypothalamus-Pituitary-Adrenocortical Axis

Limbic System
(hipp/ amyg.)  

CRH  AVP

PVN

ATII

CRH  AVP

Pituitary

ACTH

Adrenal Cortex
Cortisol, Aldosterone

GR/MR

BBB
MR, but not GR, are reduced in the hippocampus of suicide victims (Lopez et al., 1998)
Effect of Imipramine Treatment on mRNA Expression
(Brady et al., 1992)

Table I. Expression of mRNA in Rat Brain after Chronic Imipramine

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Control</th>
<th>Imipramine 2 wk</th>
<th>Imipramine 8 wk</th>
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<tbody>
<tr>
<td>Hippocampus</td>
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<tr>
<td>MR</td>
<td>100±18</td>
<td>130±31</td>
<td>170±22†</td>
</tr>
<tr>
<td>GR</td>
<td>100±5</td>
<td>96±5</td>
<td>104±5</td>
</tr>
<tr>
<td>PVN</td>
<td></td>
<td></td>
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<tr>
<td>CRH</td>
<td>100±14</td>
<td>104±11</td>
<td>63±15§</td>
</tr>
<tr>
<td>GR</td>
<td>100±24</td>
<td>73±41</td>
<td>72±33</td>
</tr>
<tr>
<td>MR</td>
<td>100±10</td>
<td>96±4</td>
<td>92±7</td>
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<tr>
<td>Pituitary, anterior lobe</td>
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<td></td>
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<tr>
<td>POMC</td>
<td>100±30</td>
<td>82±24</td>
<td>62±31*</td>
</tr>
<tr>
<td>GR</td>
<td>100±46</td>
<td>52±47</td>
<td>49±14*</td>
</tr>
<tr>
<td>MR</td>
<td>100±20</td>
<td>95±12</td>
<td>104±9</td>
</tr>
<tr>
<td>Pituitary, intermediate lobe</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>POMC</td>
<td>100±3</td>
<td>101±1</td>
<td>100±2</td>
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<tr>
<td>MR</td>
<td>100±24</td>
<td>117±32</td>
<td>130±35</td>
</tr>
<tr>
<td>Locus coeruleus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TH</td>
<td>100±18</td>
<td>80±8*</td>
<td>60±11‡</td>
</tr>
<tr>
<td>GR</td>
<td>100±15</td>
<td>90±11</td>
<td>101±16</td>
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</table>
Apparently the MR is linked to depression.

Is there a role of aldosterone?
HPA and RAAS hormones in depressed patients and healthy controls (Murck et al., 2003)

renin

aldosterone

ACTH

cortisol

p < 0.1

p < 0.05

p < 0.1
Increase in Aldosterone in patients with depression (Emanuele et al., 2005)

Figure 1. Scatter diagram for plasma aldosterone concentrations in depressed patients ($n = 65$) and in healthy control individuals ($n = 65$). Horizontal lines across the scatter diagram represent mean values.
MDE plus hypertension related to increased aldosterone levels (Haefner et al., 2011)
Is high aldosterone a biomarker or causally involved in depression?
AT1- and ACE-polymorphisms predict outcome in antidepressant response (Bondy et al., 2005)

![Graph showing the percentage reduction in HAMD-17 score from day 0 to 28 and the effect of polymorphisms in the ACE and AT1 receptor genes on clinical response.](image)

Fig. 1. Polymorphisms in the ACE and AT1 receptor genes in relation to clinical response (% reduction in HAMD-17 scale within 4 weeks of treatment) in 273 patients with major depression.
Consequence of Aldosterone Excess
(Gomez-Sanchez et al., 2011)
MR Agonist DOC suppresses nocturnal Cortisol secretion in healthy volunteers (Steiger et al., 1993)
MR-antagonism with canrenoate reduces Slow Wave Sleep (SWS) in healthy controls (Born et al., 2001)

MR activation suppresses SWS
Methods

• Observational study: baseline (at admission), 2 weeks, 6 weeks

• Patients with unipolar depression
• No relevant internistic diseases

• Parameters:
  • HAMD-6, HAMD-21, QIDS-SR; BDI
  • Salivary Aldosterone, salivary cortisol
  • slow wave sleep
  • Heart rate; heart rate variability
  • Blood pressure
  • Salt taste test
Aldosterone and Cortisol *(Jezova et al. 2008)*

- Saliva collection after awakening, patients still in bed.
- Modified RIA with concentrated saliva
Zeo-Sleep EEG
Zeo-Sleep EEG
i-thlete device
Salt taste questionnaire

Ca. 10 min. after awakening

0,9%ige NaCl solution on cotton swab

1. Mit welcher Salzigkeit würden Sie die Salzlösung auf der visuellen Skala einschätzen? (bitte ankreuzen)
(0 = keine Salz in der Lösung vorhanden; 10 = extrem hoher Anteil an Salz in der Lösung)

![Salzskala](image)

2. Wie haben Sie Salzgummschmack empfunden? (bitte ankreuzen)
(0 = extrem unangenehm; 10 = sehr angenehm)

![Gummiempfindung](image)
Aldosterone correlates with depression severity in pooled data
Aldosterone/Cortisol Ratio at baseline predicts outcome

Relative HAMD-21 at outcome

Aldosterone/Cortisol Ratio

R² Linear = 0.264

p<0.05
Cortisol reduction at week 2 determines outcome

![Graph showing the relationship between cortisol change and HAMD-21 outcome. The graph includes lines for male and female data points, indicating a significant correlation with R^2 values of 0.351 and 0.238.](image)
Time course of subjective salt-intensity perception

Subjective Salt-intensity

Visit

Timecourse of salt preference

Salt-Preference

Visit

$p<0.05$

$p<0.05$
Clinical improvement correlates inversely with HRV at baseline (male subjects)

Relative HAMD-21 at outcome

R² Linear = 0.318

HRV
Clinical improvement correlates inversely with SWS at baseline (male subjects)
Clinical improvement correlates inversely with Na\(^+\) at baseline (male subjects)
Preliminary data: MR antagonists have beneficial effect on affective symptoms

- spironolactone: decreases irritability, depression, food craving, pain, (crossover design) (Wang et al., 1995).

- drospirenone: decreases depressive mood (Yonkers et al., 2005)
Physiology is complex and biological markers require interpretation

see Murck et al., 2012, Pharmacopsychiatry
Several inexpensive and easy to handle markers exist for the characterization of mineralocorticoid-function.

MR-related parameters are predictive markers for clinical outcome.

Manipulation of the RAAS may effect clinical outcome in depression.
• Backup
Action at aldosterone-specific brain areas
Co-localization of MR and HSD2 in cells of the nucleus of the solitary tract (Geerling et al., 2006)

Fig. 3. HSD2 immunoreactivity (green) was found in the somatic and dendritic cytoplasm of NTS neurons with nuclear (red) and perinuclear (yellow) MR immunoreactivity. Scale bar = 15 μm.
Colocalized MR and 11\(\beta\) HSD
(Gomez-Sanchez et al., 2012)

<table>
<thead>
<tr>
<th>Rat brain (adult)</th>
<th>MR</th>
<th>11(\beta)-HSD2 adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Hippocampus</td>
<td>xx</td>
<td></td>
</tr>
<tr>
<td><strong>Hypothalamus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVN</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>VMN</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td><strong>Amygdala</strong></td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Thalamus</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td><strong>Brain stem</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTS</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Locus coeruleus</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Med vestibular nuc</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Subcommissural organ</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Choroid plexus</td>
<td>xx</td>
<td></td>
</tr>
</tbody>
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Conn Syndrome
Increased depression, anxiety, irritability in patients with hyperaldosteronism (Sonino et al., 2011)

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<tr>
<th>Diagnosis</th>
<th>PA (n = 23)</th>
<th>EH (n = 23)</th>
<th>Controls (n = 23)</th>
<th>P value</th>
</tr>
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<tr>
<td><strong>Psychiatric diagnoses</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anxiety disorders</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Generalized anxiety disorder</td>
<td>7 (30.4%)(^{a})</td>
<td>2 (8.7%)</td>
<td>0</td>
<td>0.007</td>
</tr>
<tr>
<td>Panic disorder</td>
<td>2 (8.7%)</td>
<td>1 (4.3%)</td>
<td>0</td>
<td>0.35</td>
</tr>
<tr>
<td>Social phobia</td>
<td>2 (8.7%)</td>
<td>1 (4.3%)</td>
<td>0</td>
<td>0.35</td>
</tr>
<tr>
<td>Agoraphobia</td>
<td>1 (4.3%)</td>
<td>0</td>
<td>1 (4.3%)</td>
<td>0.59</td>
</tr>
<tr>
<td><strong>Mood disorders</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Major depressive disorder</td>
<td>5 (21.7%)</td>
<td>4 (17.4%)</td>
<td>0</td>
<td>0.06</td>
</tr>
<tr>
<td>Cyclothymic disorder</td>
<td>1 (4.3%)</td>
<td>0</td>
<td>0</td>
<td>0.36</td>
</tr>
<tr>
<td>Dysthymic disorder</td>
<td>1 (4.3%)</td>
<td>1 (4.3%)</td>
<td>0</td>
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<td><strong>Psychological Syndromes</strong></td>
<td></td>
<td></td>
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<td>Irritable mood</td>
<td>10 (43.5%)(^{b})</td>
<td>7 (31.8%)(^{b})</td>
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<td>0.028</td>
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<td>0.34</td>
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<tr>
<td>Persistent somatization</td>
<td>2 (8.7%)</td>
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\(^{a}\) P < 0.01, PA vs. EH and controls.

\(^{b}\) P < 0.01, PA and EH vs. controls.
Subchronic aldosterone administration
- a depression model
Aldosterone-treatment resulted in increased depression-like behavior in the forced swim test in rats (Hlavacova et al., 2011)
Aldosterone-treatment induced an anhedonic state manifested by decreased sucrose preference (Hlavacova et al., 2011)
No effect on stress induced HPA-axis activity (Hlavacova et al., 2011)
Large overlap in gene expression changes in response to stress and aldosterone

- **Criteria**
  - $p<0.05$
  - $|\text{fold-change}|>1.2$
- **Stress vs. non-stressed**
  - 99 probe sets
- **Aldosterone vs. vehicle**
  - 254 probe sets
- **Gene set enrichment analysis**
  - Acute phase inflammation
  - Complement activation
- **73 genes in common**
  - 74% of genes affected by stress are also affected by aldosterone
Hippocampal gene expression changes

Hlavacova et al., 2011
Glutamatergic transmission

**G protein signaling**

**Postsynaptic Neuron**

**Homer**

**PSD-95**

Clustering of NMDA receptors

**Ca\(^{2+}\)**

**Na\(^{+}\)**

**K\(^{+}\)**

Late phase of EPSPs

Activation of Ca\(^{2+}\)-dependent enzymes and 2nd messengers

Long-lasting synaptic changes

Overstimulation causes glutamate toxicity

**EAAT4**

**SLC1A2/3**

**SLC38A1**

**Glu**

**Gln**

**Depolarization**

**Early phase of EPSPs**

**Depolarization**

**Activation of Ca\(^{2+}\)-dependent enzymes and 2nd messengers**

**Long-lasting synaptic changes**

**Overstimulation causes glutamate toxicity**

**Astrocyte**

**SLC17A**

**EAAT4**

**SLC1A1/4**

**mGluR2**

**mGluR7**

**mGluR8**

**Presynaptic Neuron**

**Synapse**

**Glu**

**Gln**

**Depolarization**

**Early phase of EPSPs**

**Activation of Ca\(^{2+}\)-dependent enzymes and 2nd messengers**

**Long-lasting synaptic changes**

**Overstimulation causes glutamate toxicity**

**Postsynaptic Neuron**
**Microarray**

*Ptgs2*

![Bar chart showing relative expression level of Ptgs2 between VEH and ALDO. The ALDO group shows a significant increase (*p* < 0.05) compared to the VEH group.*

*Faah*

![Bar chart showing relative expression level of Faah between VEH and ALDO. The ALDO group shows a significant increase (**p** < 0.01) compared to the VEH group.*

*Il1rn*

![Bar chart showing relative expression level of Il1rn between VEH and ALDO. The ALDO group shows a significant increase (*) compared to the VEH group.*

**qRT-PCR**

*COX-2*

![Bar chart showing relative expression level of COX-2 between VEH and ALDO. The ALDO group shows a significant increase (*) compared to the VEH group.*

*Fatty-acid amine hydrolase: cannabinoid metabolism*

*Interleukine Receptor antagonist*
Potential modes of aldosterone action:

1. Increased excretion of Mg\(^{2+}\), reduced brain Mg\(^{2+}\)  
   \(\Rightarrow\) increased NMDA activity

2. Activation of selective classic MR in the CNS, in particular in the nucleus of the solitary tract (NTS) (Geerling et al., 2009)

3. Activation of membrane MR (hippocampus)  
   (Karst et al., 2008)

not mutually exclusive!
Increased depression, anxiety, irritability in patients with hyperaldosteronism (Sonino et al., 2011)

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Roads to high aldosterone:

- Aldosterone is a stress hormone
- Regulation via ACTH, ACE, ATII etc. polymorphisms may affect release
- Counterregulatory stimulated by low blood pressure, low Na\(^+\), high K\(^+\).