Presentation 8 – Heremona Soreq and Steven Berkowitz

Serum enzyme activities in Gulf War Era Deployed Veterans and demographic parameters:

Progress report and comparison to healthy individuals

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Objective

To determine serum enzyme activities among an anonymous group of Gulf War Veterans as compared to healthy U.S individuals.

AChE-R: The Stress Response Culprit

Long-term dangers

- Extracellular Impairment of cholinergic homeostasis
- (Kaufer, Nature, 98)

⇨ Depression

- Disruption of synaptic interactions
- (Soreq, Nature Neur, 01)

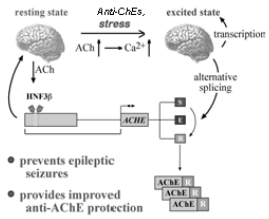
⇨ Cognitive deterioration

- Extrasynaptic functioning
- (Meshorer, Science, 02)

⇨ Hypersensitivity

- Intracellular induction of stress signal transduction
- (Birikh/Sklan, PNAS, 03)

⇨ Extended conflict behavior

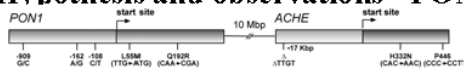


Symptoms associated with Multiple Syndromes

Hypothesis and observations - AChE

- Acetylcholinesterase (AChE) overproduction reflects organismal response to stressful stimuli or anti- AChE exposure [Kaufer et al., Nature 1998; Meshorer et al., Science 2002].
- In Healthy U.S individuals, demographic parameters were assessed and a series of equations developed in Jerusalem to predict serum enzyme levels [Sklan et al., submitted].
- In Gulf War Veterans, we observe conspicuous deviations from the predicted values.

Hypothesis and observations - PON



- Paraoxonase (PON) protects serum proteins (AChE included) from oxidative stress. In healthy individuals, we found an inverse correlation of AChE & PON with anxiety measures [Sklan et al.,submitted; Bryk et al., in preparation].
- Therefore, we further tested serum PON activities and examined their correlation to the AChE values.

Hypothesis and observations– Control enzyme assays

- Butyrylcholinesterase (BChE) is homologous to AChE. In both US and Israeli healthy individuals, AChE and BChE activities are directly correlated. We observe a different Correlation to AChE in Gulf War Veterans.
- Arylesterase (Aryl) activity reflects a distinct enzymatic activity of the PON protein (which differs from its paraoxon hydrolysing activity). Each of these activities distinctly depends on the genotype.
  In healthy individuals, Aryl activities are considerably less variable than PON activities.

Technical considerations

- The vast majority of the samples were from Caucasian males. Therefore, we excluded the other samples from most of our current data management (and used only Caucasian male samples from the healthy populations for comparison).
- Most psychoactive drugs may be expected to inhibit AChE activity and induce a feedback response of AChE overproduction, while suppressing liver metabolism and reducing PON and BChE activities.

Conclusions

- Gulf War Veterans display massive AChE overproduction.
- Deviations from the equation developed for healthy individuals calls for comparative genotype study to explore inherited origin for the discovered differences.
- Serum enzyme activities among Gulf War Veterans differ from those of healthy individuals in U.S. and Israel by several criteria.
- Genotype information will be required to develop serum PON activities, as surrogate measure of symptoms.

Conclusions – Control enzyme assays

- AChE and BChE activities are directly correlated both in Gulf War veterans and in healthy individuals. This correlation is less pronounced when BMI or age considerations are made , attributing much of the inter-individual variability to weight and age origins.
- In Gulf War veterans, Arylesterase activities show direct and significant correlation to BMI.

Acetylcholinesterase (AChE) Activity in Gulf War Deployed and Era Veterans

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Background

- Approx. 700,000 US troops were deployed to the Gulf during Operation Desert Shield/Desert Storm
- Numerous deployed veterans reported multiple illness symptoms, (unexplained fatigue, pain, reduced cognitive function, and other symptoms)
- There is a great need to discover a biological mechanism or cause, in order to identify effective treatments for our veterans affected by GWVI

Neurotransmitters

- Acetylcholine (ACh)-mediated neurotransmission is fundamental for nervous system function.
- Loss of ACh function, is assoc. with deterioration of cognitive, autonomic & neuromuscular functions
- AChE hydrolyses/inactivates ACh, regulating the concentration of the ACh at the synapse
- Battlefield and environmental exposures can increase AChE transcription thereby decreasing the action of ACh

Exposures

- During the war, soldiers were exposed to harsh climate, anti-AChE insecticides, numerous vaccinations, and battlefield experiences.
- An anti-AChE, pyridostigmine bromide, was given as a prophylactic against chemical warfare
- In short-term peacetime tests, pyridostigmine bromide was deemed safe for the troops, however, it's long-term effects are not known.

Potential Relationships

- Preliminary research suggested a possible role of AChE, Butyrylcholinesterase (BuChE) and Paroxynase (PON), and environmental exposure, as factors in neurological symptoms/muscle weakness
- Animal studies found that stress increases lethality of low levels of an anti-AChE pesticides, suggesting the potential link between stress and AChE activity
- Exposure to anti-AChEs, combined with battlefield experiences, may have contributed potentially to the fatigue, pain, and memory symptoms in GWVI

Biological Mechanisms

- Previous findings are consistent with the idea that stress-induced neuronal signal transduction involves AChE-R overproduction.
- These findings suggest interaction effects with drug responses and exposure to certain chemical compounds that modulate ACh neurotransmission
- Such agents include insecticides (e.g. chlorpyrifos) and therapeutic anticholinesterases).

AChE and Anxiety

- The physiological stress response is expressed primarily in the body's endocrine and neural systems.
- Analysis of AChE activity in Blood samples from 470 individuals (from a normal US population) found a strong correlation with both acute (state) and baseline (trait) anxiety levels.
- As serum AChE levels decreased, the reported levels of anxiety decreased

Objective & Hypotheses

- To determine if mood/anxiety symptoms are related to serum levels of AChE, BuChE, and/or PON among Gulf War deployed and era veterans.
- **Hypotheses:**
  - Serum AChE levels are associated with mood and anxiety symptoms, particularly anxiety.
  - Deployed Gulf War veterans have lower Blood AChE levels than non-deployed.
  - Serum AChE-R ("Readthrough" variant) levels are associated with mood and anxiety symptoms

Study Design

- **Designed to determine any association between blood levels of AChE, BuChE, and PON, and mood/anxiety symptoms in Gulf War era veterans.**
  - Laboratory analysis of stored serum samples will determine levels of AChE, BuChE, and PON among 572 participants in the Iowa Gulf War Cohort Study
  - Laboratory test results will be linked to the health data obtained in the Iowa Gulf War Study and then analyzed to discover any associations that may exist.

Preliminary Data

- Initial analyses have been completed on a portion of the Iowa Gulf War Study blood samples.
- Description of Preliminary Data
  - **Dr. Hermona Soreq**