Novel Molecular Mechanisms for Neurological Morbidities in a DFP Based Rat Model of Gulf War Illness

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INTRODUCTION

Chronic exposures to organophosphates (OP) including pesticides and nerve gas during the Persian Gulf War has been attributed to the development of a debilitating disorder known as Gulf War Illness (GWI)[1,2]. Despite current treatment recommendations, GWI Veterans continue to suffer from neurological morbidities of depression, and memory impairments amongst others[3].

We have developed an OP-based rat model of GWI that mimics these neurological symptoms in the absence of other confounding factors present in the war theatre[4]. This model is being used to identify molecular correlates for GWI to develop effective treatment solutions. A molecular mechanism that is commonly altered in neurological disorders is aberrant calcium signaling.

Calcium ions (Ca^{2+}) are signaling molecules modulating memory, mood, and behavior functions. Disruptions in neuronal Ca^{2+} are implicated in Alzheimer's, Epilepsy, TBI, conditions which manifest similar neurological morbidities as seen in GWI[5]. However, the status of Ca^{2+} homeostasis in the development of behavioral impairments in GWI is unknown, and thus is the focus of this investigation.

METHODS

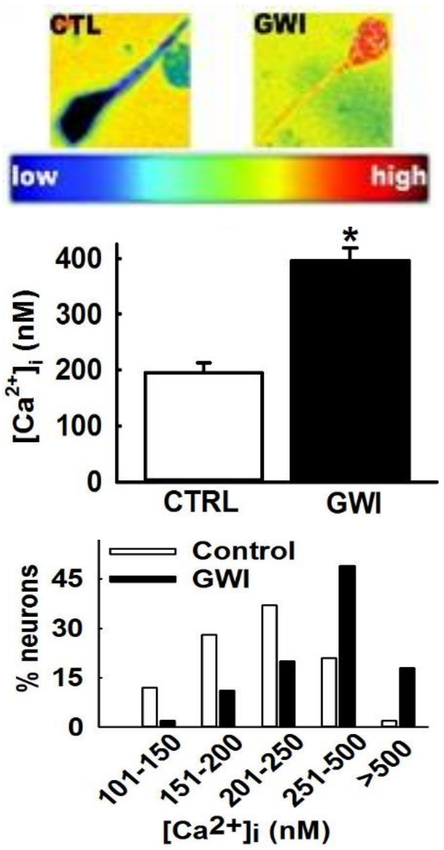
GWI Model: Male rats (S.D., 9-weeks) were injected once daily with DFP (0.5 mg/kg, s.c., ice-cold PBS) for 5-days, while control rats received saline injections[5]. At 3-months post-DFP exposure, rats were tested for the symptoms of depression and cognitive deficits using a battery of behavioral assays as described earlier[4].

Calcium Imaging: CA1 neurons were acutely isolated from hippocampal slices and loaded with fluorescent Ca^{2+} indicator Fura-2AM. They were then stimulated using alternating 340/380 wavelengths and resulting emissions were acquired to record Ca^{2+} transients as described earlier[5].

Calcium Calibration: Calibration curve was constructed using solutions of calibrated Ca^{2+} buffers ranging from Ca^{2+} free to 39 μM Ca^{2+}. Values from the calibration curve were used to convert fluorescent ratios to $[Ca^{2+}]_i$. Final $[Ca^{2+}]_i$ were calculated from the background corrected 340/380 ratios using the Grynkiewicz eq^n: $[Ca^{2+}]_i = (K_d \times Sf_2 / Sb_2) \times (R - R_{min}) / (R_{max} - R)$

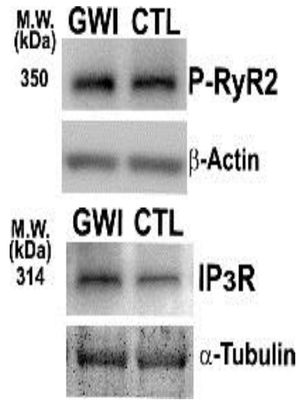
Western Blotting: Hippocampal tissue from GWI rats processed using standard procedures. From each rat, hippocampal homogenates were prepared. Quantitation of the amount of RyR-p, IP3-R, and PLCγ per mg protein in each sample was performed. Antibody specificity was established using blocking peptides and no antibody controls. Internal and external standards to control for loading and sample variability were used.

Elevated hippocampal $[Ca^{2+}]_i$ in GWI neurons



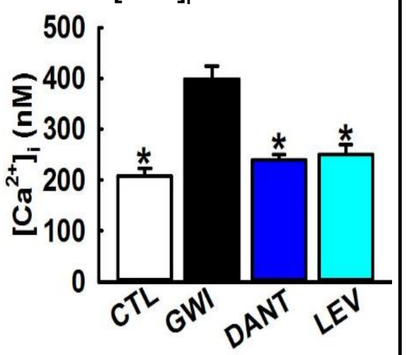
Acutely isolated CA1 neurons from GWI rats manifested $[Ca^{2+}]_i$ of 399 ± 26 nM, that were higher than $[Ca^{2+}]_i$ in neurons from age-matched controls (208 ± 16 nM, n= 7 animals, p<0.05, t-test). Analysis of the population distributions of $[Ca^{2+}]_i$ revealed a right-ward shift towards higher Ca^{2+} concentrations in GWI neurons compared to control neurons (p<0.01, ψ^{-2} test, n= 161 neurons).

Increased CICR protein expression in GWI rats



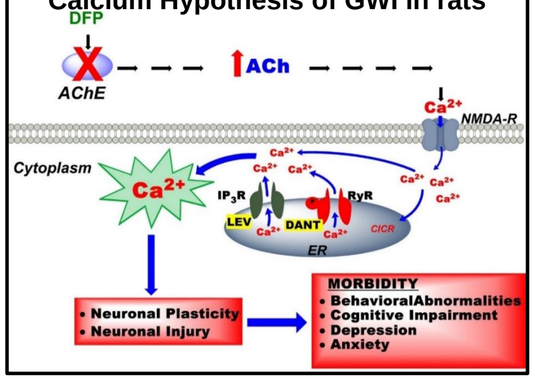
Western blot analysis showed increased levels of intracellular Ca^{2+} release receptor proteins P-RyR_2 (100 ± 14.2%, 124.2 ± 31.9%) and IP_3-R (100.0 ± 48.6%, 105.8 ± 43.2%).

CICR antagonists lower elevated $[Ca^{2+}]_i$ in GWI neurons



Dantrolene (DANT, 50 μM) or levetiracetam (LEV, 100 μg) lowered elevated $[Ca^{2+}]_i$ in GWI neurons (240 ± 11 nM and 250 ± 19 nM respectively, n= 5 animals, p<0.05, t-test).

Calcium Hypothesis of GWI in rats



CONCLUSIONS

We observed that GWI rats manifested chronic elevations in hippocampal Ca^{2+} levels. The protracted Ca^{2+} elevation had its origin in Ca^{2+} release from intracellular Ca^{2+} stores, since the ryanodine/IP_3 receptor antagonists produced a significant reduction in elevated $[Ca^{2+}]_i$ in GWI neurons. These sustained $[Ca^{2+}]_i$ elevations appear to be due an increased expression in major components of intracellular Ca^{2+} release machinery, particularly the RyR_2 receptor. Mechanisms underlying increased expression of P-RyR_2 are being investigated.

Since Ca^{2+} is a major second messenger molecule, such chronic increases in its levels could activate signaling cascades that could underlie the neuronal damage and also produce pathological synaptic plasticity that expresses itself as GWI morbidity. Treatment with drugs targeted at blocking this enhanced intracellular Ca^{2+} release could be effective therapies for GWI related neurological morbidities.

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