Effect of Basal Ganglia Injury on Central Dopamine Activity in Gulf War Syndrome

Correlation of Proton Magnetic Resonance Spectroscopy and Plasma Homovanillic Acid Levels

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Background: Many complaints of Gulf War veterans are compatible with a neurologic illness involving the basal ganglia.

Methods: In 12 veterans with Haley Gulf War syndrome 2 and in 15 healthy control veterans of similar age, sex, and educational level, we assessed functioning neuronal mass in both basal ganglia by measuring the ratio of N-acetyl-aspartate to creatine with proton magnetic resonance spectroscopy. Central dopamine activity was assessed by measuring the ratio of plasma homovanillic acid (HVA) and 3-methoxy-4-hydroxyphenlyglycol (MHPG).

Results: The logarithm of the age-standardized HVA/MHPG ratio was inversely associated with functioning neuronal mass in the left basal ganglia ($R^2=0.56; F_{1,27}=33.82; P<.001$) but not with that in the right ($R^2=0.04; F_{1,26}=1.09; P=.30$). Controlling for age, renal clearances of creatinine and weak organic anions, handedness, and smoking did not substantially alter the associations.

Conclusions: The reduction in functioning neuronal mass in the left basal ganglia of these veterans with Gulf War syndrome seems to have altered central dopamine production in a lateralized pattern. This finding supports the theory that Gulf War syndrome is a neurologic illness, in part related to injury to dopaminergic neurons in the basal ganglia.

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During and immediately following the Persian Gulf War, large numbers of veterans developed troublesome symptoms such as problems with attention and memory, depression, personality change including difficulty controlling anger, constant body pain, stuttering, chronic diarrhea, and attacks of vertigo.1,3 Early reports of slightly elevated scores on the Mississippi Post-Traumatic Stress Disorder (PTSD) Scale led to a widespread attribution of the symptoms to combat-related PTSD.4 However, the low specificity of the measurements used for PTSD, the small numbers of PTSD cases eventually confirmed by psychiatrists in structured clinical interviews, and the lack of correlation with combat exposure scales suggest that PTSD was relatively rare in Gulf War veterans and that the symptoms of Gulf War syndrome were probably caused by something other than stress.

Some of the symptoms of Gulf War syndrome resemble those in the early, presenting stage of primary degenerative diseases of basal ganglia, such as Huntington, Wilson, and Fahr diseases.6 Thus, I and another team of coworkers undertook a clinical case-control study using magnetic resonance (MR) spectroscopy to examine basal ganglia function in Gulf War veterans meeting Haley’s previously published case definition and in matched control veterans. The results showed a reduction of functioning neuronal mass in the basal ganglia and brainstem of these subjects vs controls.9 The prior clinical and epidemiologic studies suggest that the neuronal injuries resulted from exposure to combinations of neurotoxic chemicals in those military personnel with a genetically determined susceptibility to organophosphates such as chemical nerve agents.

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We here extend the study of basal ganglia function with measurements of an index of central dopamine activity in the same group of Gulf War syndrome subjects and controls. The purpose of the extended study is to determine whether the reduction in functioning neuronal mass of either basal ganglia is associated with abnormalities of central dopamine activity.
SUBJECTS, MATERIALS, AND METHODS

SUBJECTS

In a previous survey of symptoms that began during or shortly after the Gulf War in 249 members of a Naval reserve construction battalion, factor analysis of symptoms was used to identify 3 primary and 3 secondary symptom complexes as possible variants of Gulf War syndrome.1 To examine a possible neurologic basis for the symptom complexes, typical cases of each primary symptom complex were selected (5 of complex 1, 13 of complex 2, and 5 of complex 3) as well as 20 age- and sex-matched controls who had remained healthy.10 Ten of the controls had served in the Gulf War, and 10 were in the same battalion but were not deployed to the war zone. Neurpsychological, audiovestibular, and neurophysiologic testing indicated that the veterans with the symptom complexes had greater organic neurologic dysfunction than the controls.10 The veterans with symptom complex 2 had the most severe audiovestibular dysfunction and were clinically more impaired, as indicated by a higher prevalence of unemployment and higher scores on the Halstead-Reitan neuropsychological battery.11,10 Subsequent studies in the same case and control veterans found the case veterans to have lower blood levels of PON1 type Q paraoxonase/arylesterase (PON-Q), a genetically regulated enzyme that hydrolyzes the chemical nerve agent sarin,12 and evidence of lower functioning neuronal mass in the basal ganglia and brainstem than controls.9 On all of these measures, the veterans with symptom complex 2 seemed to be more severely affected than those with the other 2 primary symptom complexes.3,10,12

For the present study, we included 27 veterans from the prior case-control study, of whom 12 had Gulf War syndrome of Haley symptom complex 2 (“confusion-ataxia”)9; 8 controls served in the Gulf War but remained healthy; and 7 did not serve in the Gulf War and also remained well. All of the subjects were white men, and the 3 groups were similar in age (mean ages, 51.8, 50.3, and 50.4 years, respectively), education level (mean years of education, 12.6, 12.2, and 13.4, respectively), and renal function (mean creatinine clearance, 2.20 (132), 2.17 (130), and 1.87 (112) mL/s (mL/min), respectively). Twenty-five subjects were right-handed; whereas, 1 case veteran and 1 control were left-handed. Ten case veterans and 11 controls smoked. All subjects gave written informed consent after the nature of the procedures had been fully explained, and the research protocol was approved by the institutional review board of the University of Texas Southwestern Medical Center at Dallas.

CLINICAL RESEARCH PROTOCOL

After stopping treatment with potentially interfering medications at least 3 half-lives before arrival, the subjects were admitted to a metabolic research unit of our hospital for 7 days during which they maintained a sedentary, low-stress activity level and received a uniformly high (8 g/d) sodium diet to avoid volume depletion.13 On the first day, each subject underwent MR spectroscopy. On the sixth day, venous blood was drawn at 7:30 AM by an experienced phlebotomist after the subjects had been fasting for 14 hours. Blood specimens were taken for routine clinical tests including serum creatinine, and additional blood specimens were immediately cooled in ice water, centrifuged, and the plasma frozen for later assay.

MR SPECTROSCOPY

Subjects underwent long echo time (TE=272 ms) MR spectroscopy of the brain to estimate intracellular concentrations of N-acetyl-aspartate (NAA), choline (Cho), and creatine (Cr) in a 4 × 2 × 2-cm voxel in each basal ganglia and a 2 × 2 × 2-cm voxel in the pons. N-acetyl-arylesterase, one of the most abundant chemicals in the brain, is primarily localized to the cytoplasm of neurons and decreases, sometimes reversibly, with most disease processes that injure or kill neurons.13 Creatine, involved in energy metabolism and generally unaffected by disease processes, is used for standardizing NAA and Cho measurements for comparing individuals or groups.13

Data were acquired on a MR imaging/MR spectroscopy scanner operating at 1.5 T (Philips Gyroscan NT; Philips Medical Systems, Best, the Netherlands), using a 30-cm-diameter head coil for both excitation and reception of the proton MR signal. The postprocessing of the spectra was automated16 and performed in the time domain by an analyst who was blinded to the subjects' clinical status. Hankle single-value deconvolution filtering was used to remove artifacts.

The age-standardized central dopamine index was inversely associated with the functioning neuronal mass of the left basal ganglia (F1,17=33.82; P<.001), but it was not associated with the functioning neuronal mass of the right basal ganglia (F1,16=1.09; P=.31; Figure). In a multiple regression analysis, the strength of the association between the central dopamine index and the NAA/Cr ratio in the left basal ganglia was not appreciably affected by controlling for the subjects' age, overall renal function (creatinine clearance), renal clearance of weak organic anions (plasma 5-HIAA concentration), handedness, and smoking history (Table). Of these potential confounding variables, only age remained a significant predictor of the central dopamine index in the adjusted regression model (Table). The association between the age-standardized central dopamine index and the NAA/Cr ratio...
residual water signal in the time domain. The resonance intensities (concentration estimates) of NAA, Cho, and Cr in each volume of interest were estimated with the FITMasters program (Fast Interpretation of Time Domain Data by Multi-component Analysis of Selectively Truncated Exponential Resonance Signals; Philips Medical Systems), which automatically fits separate sine waves to the NAA, Cho, and Cr peaks of the acquired spectra in the time domain to estimate the integral (amplitude in the time domain), frequency, and T2 time constant. Chemical shifts were calculated relative to the NAA peak at 2.01 ppm. For model fitting, the frequencies of the peaks for NAA, Cho, and Cr were fixed at 2.01, 3.02, and 3.20 ppm, respectively, and their 1/T2 values were constrained to vary together at 8.84, 5.86, and 4.05 Hz, respectively. Intensity fitting was unconstrained. Further details of the imaging protocol and findings have been published.

ASSAYS

High-performance liquid chromatography was used to measure plasma levels of homovanillic acid (HVA), a dopamine metabolite, 3-methoxy-4-hydroxyphenylglycol (MHPG), a norepinephrine metabolite, and 5-hydroxy-3-indoleacetic acid (5-HIAA), a serotonin metabolite. The assay coefficients of variation for these assays were 8.7%, 4.8%, and 12.0% for HVA, MHPG, and 5-HIAA, respectively. We also collected 24-hour urine samples on 3 consecutive days during hospitalization for measurement of creatinine levels and urine volume.

CENTRAL DOPAMINE INDEX

To obtain a measure of central dopaminergic activity correcting for contributions from peripheral catecholamine metabolism, we calculated a central dopamine index defined as the ratio of plasma levels of HVA and MHPG. Described by Ottong and Garver, this index is a simple extension of the observation of Kopin et al and Amin et al that in normal primates and humans receiving increasing doses of debrisoquin to block peripheral catecholamine metabolism, the association between plasma HVA and MHPG levels fits a straight regression line with a positive slope and a y intercept (extrapolated to 0 MHPG) that closely approximates dopamine activity in the brain. Given this relationship, the simple plasma HVA/MHPG ratio represents an index of central dopamine activity or metabolism at constant MHPG on which groups of subjects can be compared. Because of the known decrease of central dopamine activity with age, we created an age-adjusted version of the index by dividing the plasma HVA/MHPG ratio by age (in years) for graphical analyses.

OTHER METABOLIC INDICES

Although we ensured a constant euovolemic state in all subjects, we used plasma 5-HIAA levels to examine whether differences in plasma HVA levels were potentially influenced by differences in selective renal excretion of weak organic anions. An estimate of glomerular filtration rate was calculated on 3 consecutive 24-hour urine collections from the product of the urine creatinine (mg/dL) and the 24-hour urine volume (milliliters) divided by the product of serum creatinine (mg/dL) and duration of the urine collection (minutes). The values for a given day were excluded if the total creatinine clearance per body weight in the sample was less than 1.5 mg/kg. The maximum of the remaining daily values for creatinine clearance was used as the measure of glomerular filtration rate.

DATA ANALYSIS

We tested the difference in the NAA/Cr ratio in the case and control groups in both the left and right basal ganglia with repeated-measures analysis of variance. We tested the laterality hypothesis by regressing the natural log transformation of the age-standardized central dopamine index on the NAA/Cr ratio of each basal ganglia. To test the possibility of confounding by factors potentially not entirely controlled by the experimental conditions, we retested all statistically significant results in multiple regression models controlling for age, creatinine clearance, renal clearance of weak organic anions (5-HIAA), handedness, and history of smoking. For each regression analysis, we examined the scatterplot for nonlinear associations and, when indicated, performed appropriate transformations to linearize the bivariate distributions for final regression analyses. Statistical tests were performed with the correlation, regression, and general linear procedures of SAS (version 6.12; SAS Institute, Cary, NC).

Our finding of a strong association between reduced functioning neuronal mass in basal ganglia (measured by MR spectroscopy) and central dopamine activity (measured by plasma HVA) adds biological plausibility to our previous finding of reduced functioning neuronal mass in the basal ganglia of some veterans with Gulf War syndrome. The original MR spectroscopy finding of significantly reduced NAA/Cr ratio in the basal ganglia of veterans satisfying Haley’s case definition of Gulf War syndrome 2 (confusion-ataxia) compared with matched controls, though suggestive of illness or injury affecting basal ganglia, could potentially be explained by other factors, such as undetected differences in partial-volume effects between cases and controls, other unknown methodologic differences, or unusual sampling variation in a case-control study with a relatively small sample size. The fact that our study was the first to examine the hypothesis of basal ganglia damage as the basis for Gulf War syndrome contributes further to uncertainty in the interpretation of the result. However, demonstrating a plausible neurotransmitter derangement strongly associated with the neuronal abnormality of basal ganglia adds weight to the etiologic interpretation implicating damage to striatal dopaminergic neurons in these Gulf War veterans.

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The finding that central dopamine activity was strongly associated with reduced functioning neuronal mass in the left basal ganglia, but not with the similar reduction in the right basal ganglia, is compatible with the laterality in control of central dopamine activity demonstrated previously in rodent experiments. Dopaminergic systems, known to be distributed asymmetrically between the hemispheres of the brain, are thought to contribute to behavioral asymmetries such as handedness in humans and turning preference in animals. The asymmetry involves not only interhemispheric differences in dopamine content, receptors, and metabolism, but also asymmetrical response of dopamine activity to unilateral lesions of dopaminergic pathways.

Experiments in rats have shown that unilateral destructive lesions of dopaminergic pathways in the left hemisphere sharply increase dopamine metabolism in both hemispheres, whereas unilateral lesions on the right have less effect. For example, under stereotaxic control Louilot and Choulli infused 6-hydroxydopamine (6-OHDA) into either the left or right entorhinal cortex of rats to create a selective dopaminergic lesion and 5 (6-OHDA) into either the left or right entorhinal cortex of rats to create a selective dopaminergic lesion and 5-

Multiple Regression Analysis Predicting the Central Dopamine Index by Functioning Neuronal Mass in the Left Basal Ganglia and Potential Confounding Variables

<table>
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<th>Predictor Variable</th>
<th>F_{1,22}</th>
<th>P</th>
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<td>Functioning neuronal mass</td>
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<td>.20</td>
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<tr>
<td>Smoker</td>
<td>0.05</td>
<td>.90</td>
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*Model R^2 = 0.63; F_{1,22} = 6.18; P < .001. The central dopamine index is indicated by the ratio of plasma homovanillic acid to 3-methoxy-4-hydroxyphenylglycol; the functioning neuronal mass, by the ratio of N-acetyl-aspartate to creatine determined by magnetic resonance spectroscopy. 5-HIAA indicates 5-hydroxy-3-indoleacetic acid.

sitivity of postsynaptic D2 receptors due to loss of their dopaminergic innervation. In experimental animals, demonstration of this laterality requires production of destructive brain lesions. Thus, this laterality effect has been difficult to evaluate in humans. In the Gulf War veterans with Haley syndrome, the degree of neuronal loss in the 2 hemispheres was relatively uncorrelated. Hence, the laterality of the effects of neuronal damage upon central dopamine activity could be tested in these subjects.

Magnetic resonance spectroscopy, used widely in basic chemistry since the 1950s, has become a standard research and diagnostic tool in neuroscience and clinical practice for detecting intracellular abnormalities in brain diseases where standard MR imaging is not able to detect structural distortions. It has been widely applied to detect biochemical brain abnormalities underlying such conditions as epilepsy, Alzheimer disease, hepatic encephalopathy, multiple sclerosis, and several psychiatric diseases. The degree of difference in functioning neuronal mass between cases and controls found in this study is comparable to that reported in these brain diseases.

Likewise, the central dopamine index, calculated as the ratio of plasma HVA to MHPG, is a standard measure of dopamine metabolism in the central nervous system. In dopaminergic neurons of the brain, dopamine serves as a neurotransmitter before being metabolized to HVA. In noradrenergic neurons in both the central and peripheral nervous systems, dopamine is mostly converted to norepinephrine, which, after serving as a neurotransmitter, is metabolized to MHPG or epinephrine. A small proportion of peripheral dopamine, however, escapes metabolism through this route and is converted to HVA in the peripheral circulation, thus complicating the interpretation of plasma HVA.

Adjusting the plasma HVA concentration for MHPG largely corrects for the contribution to total plasma HVA from peripheral sources and provides a valid and reliable index of central dopamine activity. For example, Ottong and Garver found that the plasma HVA/MHPG ratio classified schizophrenic patients into high and low dopaminergic groups differently from plasma HVA alone and was more useful in predicting their re-
Measurements of plasma HVA concentrations should be made under carefully controlled conditions to ensure reliable and consistent data. A diet rich in tyrosine or tyramine can substantially elevate HVA levels immediately after the meal, but this effect dissipates after a 14-hour fast before phlebotomy.30,31 Other factors such as circadian variation, body position, vigorous physical exercise, and smoking immediately before phlebotomy can also alter plasma HVA levels, but clinical protocols for minimizing these effects have been established.39,40,42 As a result, when performed under carefully controlled conditions such as those we used, the plasma HVA/MHPG ratio can be a valid and useful measure of central dopamine activity or turnover.

The association of the plasma HVA/MHPG ratio with neuronal loss in the left hemisphere, but not with that in the right, is likely to represent a real physiologic effect for several reasons. First, the association is very strong ('Figure'). Second, the left hemispheric laterality is predicted by unilateral striatal ablation studies in rats.34,35 Third, factitious differences among subjects were minimized by the uniformity of conditions we were able to produce during a week's hospitalization in the metabolic research unit of the General Clinical Research Center at the University of Texas Southwestern Medical Center at Dallas. Subjects discontinued treatment with medications before arrival. For 5 days before phlebotomy, subjects led a nonstressful sedentary life with a uniformly high-sodium (8 g/d) diet. All blood samples were drawn uniformly at 7:30 AM to minimize differences from the known circadian fluctuations of plasma HVA levels and after a 14-hour fast to avoid effects of diet on HVA.29,40,42

Potential differences that we could not avoid included age, general renal function, specific renal clearance rates of weak organic anions, handedness, and smoking. Dopamine content of the brain declines with age.27 Plasma HVA levels can be artifically increased by reduced renal function, a selective reduction in clearance of weak organic anions, or decreased renal excretion from extracellular volume depletion.30 Laterality measurements could be confused by differences in handedness of subjects. We ruled out these possible confounding effects by controlling for them in a multiple regression analysis, and the association with functioning neuronal mass of the left basal ganglia remained strong. Only age exerted an important independent effect on the analysis.

Our findings from this study add an important new insight into an emerging theory about the nature and causes of the broad array of symptoms known as Gulf War syndrome. A series of case-control studies in the same sample of veterans has demonstrated a multisymptom-like structure to the symptoms,3 neurophysiologic evidence of organic brain dysfunction,10,43 and chemical abnormalities of neurons in basal ganglia and brainstem now associated with abnormal dopamine metabolism. Moreover, the degree of these abnormalities closely parallels the clinical severity of the symptom complexes (worse abnormalities in symptom complex 2 and less severe ones in symptom complexes 1 and 3).3,9,10,12

The etiologic connection of the syndromes with damage to the basal ganglia and brainstem is strengthened by the similarity of the symptom complexes to the early presenting symptoms of the well-known primary degenerative diseases of basal ganglia, including Huntington, Wilson, and Fahr diseases.8

The evidence further identifies specific genetic and environmental correlates that may have interacted to produce the broad clinical expression of the illness reflected by the symptom complexes. Compared with controls, the veterans with symptom complex 2 had the lowest plasma levels of PON-Q, the genetically controlled polymorphic form of an enzyme with relatively specific hydrolytic activity against the chemical nerve agent sarin; whereas, those veterans with symptom complexes 1 and 3 had intermediate reductions in PON-Q plasma levels.12 Because of the steep dose-response curves for organophosphate neurotoxicity, soldiers with blood levels of PON-Q below a critical threshold would be expected to show rapidly increasing susceptibility to low-level sarin and possibly other organophosphate toxins.

In addition, our epidemiologic studies in the 249 members of the battalion from which the cases and controls were selected identified different environmental chemical exposure profiles for the 3 primary symptom complexes.11 Symptom complex 2 was strongly associated (relative risks 4-8 and a synergistic effect) with proximity to organophosphate nerve gas alarms on a day when sarin was documented in ambient air, as well as with having experienced advanced adverse effects after taking pyridostigmine bromide tablets (used prophylactically as a soman antidote). Symptom complex 1 was strongly associated with wearing pet flea collars containing neurotoxic pesticides; and symptom complex 3, with wearing highly concentrated diethyl-toluamide (DEET) insect repellents and having experienced advanced adverse reactions to the pyridostigmine tablets. Prior toxicologic experiments in rodents have demonstrated that organophosphate toxins attack cholinesterase, neurotoxic esterase, dopamine receptors, γ-aminobutyric acid receptors, and cholinergic muscarinic receptors preferentially in basal ganglia.44 It is therefore plausible that interactions of these genetic and environmental influences caused damage to different, overlapping areas of the deep brain structures identified, leading to the varied clinical expression observed.

Our findings do not seem to support the theory that the chronic symptoms of Gulf War syndrome resulted from traumatic psychological stress.4 To our knowledge PTSD has not been associated with basal ganglia or brainstem damage or with abnormal dopamine activity or metabolism.

While preliminary and in need of replication, our findings are of primary interest in developing a better understanding of the pathophysiology of the symptoms of Gulf War veterans, but they are also important for their implications regarding the question of the laterality of dopamine control in humans. They suggest that the activity of postsynaptic dopaminergic neurons in the brain is increased in those subjects with the greatest amount of neuronal loss. This raises the possibility of future parkinsonlike syndromes in Gulf War veterans if the dopaminergic system becomes further damaged or depleted.
On the positive side, the findings suggest that measurements of functioning neuronal mass by MR spectroscopy and of central dopamine function by peripheral blood testing offer the prospect of objective measures of brain dysfunction to augment the measurement of symptom complexes used in the study of Gulf War syndrome.\textsuperscript{3,9-12} Finding an abnormality of dopamine metabolism underlying the disorder may also offer new directions for pharmacologic approaches to alleviating the often incapacitating symptoms.

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