Acetylcholinesterase inhibitors and Gulf War illnesses

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Increasing evidence suggests excess illness in Persian Gulf War veterans (GWV) can be explained in part by exposure of GWV to organophosphate and carbamate acetylcholinesterase inhibitors (AChEis), including pyridostigmine bromide (PB), pesticides, and nerve agents. Evidence germane to the relation of AChEis to illness in GWV was assessed. Many epidemiological studies reported a link between AChEi exposure and chronic symptoms in GWV. The link is buttressed by a dose–response relation of PB pill number to chronic symptoms in GWV and by a relation between avidity of AChEi clearance and illness, based on genotypes, concentrations, and activity levels of enzymes that detoxify AChEis. Triangulating evidence derives from studies linking occupational exposure to AChEis to chronic health symptoms that mirror those of ill GWV. Illness is again linked to lower activity of AChEi detoxifying enzymes and genotypes conferring less-avid AChEi detoxification. AChEi exposure satisfies Hill's presumptive criteria for causality, suggesting this exposure may be causally linked to excess health problems in GWV.

Gulf War veteran | pyridostigmine | pesticide | sarin | organophosphate

Persian Gulf War veterans (GWV) from the 1990–1991 conflict have a higher prevalence of chronic multisymptom health problems than either nondeployed personnel or those deployed elsewhere. The illness profile is reflected by higher rates of most assessed symptoms with no one symptom common to all (1). Fatigue, mood-cognitive, and musculoskeletal symptoms are often involved. The presumptive Centers for Disease Control and Prevention (CDC) definition for Gulf War illness requires chronic symptoms in two or more of those three domains (2). Similar proportions of GWV and nonGWV report low levels of assessed symptoms, with the excess in GWV comprising those with moderate, severe, or multiple symptoms within a symptom category (1). Thus, the more discriminating Kansas definition for Gulf War illness requires multiple or at least moderately severe symptoms in three or more of six symptom groups, focused on fatigue/sleep, pain, neurological/cognitive/mood, gastrointestinal, respiratory, and skin problems (1).

In epidemiological studies, 26–32% of personnel deployed to the Persian Gulf have chronic health problems after subtracting the fraction of nondeployed personnel with such problems (1, 3, 4). [This may understate the percentage affected, because troops selected to deploy to high-threat areas may have had better health than those not selected, rather than similar health (5).] This suggests that 175,000–210,000 among ≈700,000 deployed U.S. troops in excess of expectation may have chronic health problems. Some who were not deployed (or were deployed elsewhere) also report chronic symptoms. However, the rate and pattern of symptom reporting are different: a larger number of GWV report symptoms and GWV report a larger number of symptoms and greater symptom severity (1). The cause of this excess illness remains unresolved. Multiple studies now show that stress and psychological factors are inadequate to account for excess illness in GWV. Those who experienced stress and developed posttraumatic stress disorder (PTSD) clearly have elevated rates of illness. Yet, although PTSD rates are not systematically higher in GWV than in those deployed in other conflicts (6), the rate of chronic fatigue, chronic multisymptom health problems, and perceived poor health is significantly greater in the Gulf-deployed cohort (4, 7). Because the ground war lasted only 4 days, comparatively few GWV were exposed directly to combat or combat-related stressors. Health problems are common after conflicts and can arise from factors ranging from malnutrition to infectious disease, PTSD, and traumatic brain injury. Fatigue, for instance, is a sequela of many exposures and part of the health picture after many conflicts. However, the pattern and timecourse of symptoms in GWV are distinct. Musculoskeletal symptoms were not prominent in other postwar syndromes, and emergence of symptoms over several years (8) followed by symptom persistence (7) contrasts with some conflicts where symptoms reportedly resolved over time upon return. Additionally, symptom reporting is greater among Gulf-deployed personnel than among those deployed elsewhere, such as Bosnia (4).

Many GWV were exposed to organophosphate (OP) and/or carbamate acetylcholinesterase inhibitors (AChEi). (*i*) An estimated 250,000 received the carbamate pyridostigmine bromide (PB) as a nerve agent pretreatment adjunct (9). (*ii*) Pesticides, prominently including carbamate and OP pesticides, were aggressively used in an effort to control vector-borne disease, and the Department of Defense (DoD) has estimated at least 41,000 service members may have had overexposure to pesticides (10, 11). (*iii*) The DoD estimates that ≈100,000 personnel were possibly exposed to low levels of sarin nerve agent after the Khamisiyah munitions depot demolition (12) [although exposure levels and relevance are sensitive to model assumptions and have been challenged (13)]. Modeling has focused on Khamisiyah, but other nerve agent exposures may have occurred (13).

Moreover, the primary symptoms reported by ill GWV, such as fatigue, musculoskeletal, cognitive, gastrointestinal, sleep, and dermatological problems (1, 2, 4), arise in domains governed by central and peripheral cholinergic systems (systems affected by AChEis).

A 1999 RAND report articulated the possibility of a connection between AChEis and illness in GWV, including potential mechanisms (such as cholinergic dysregulation), and outlined a research approach to confirm or refute an association (9). Chapters on OP and carbamate pesticides in a subsequent report extended this (14). A number of studies have been conducted along the lines proposed, providing an opportunity for an updated assessment of the evidence.

Results

Epidemiology. Most studies examining the relation of chronic symptoms to exposures that included AChEis report a significant connection between AChEi exposure and chronic illness, despite diverse adjustment models (Table 1). Across studies, significant positive relationships of AChEi-related exposures to illness in GWV outnumber significant negative relationships more than chance would predict. The results may be influenced by self-report bias (bias is a concern to which all aggregated analyses are vulnerable); however, the studies show high consistency, with most showing a significant (typically strong) positive association. Few

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Table 1. Summary of epidemiological studies with evidence on link between at least one AChEi and chronic health problems

Author	PB*	Pesticide†	Nerve agent‡
Australian 2003 (15)	+	+	+
Bell (small N) (16)§	NS	+	NS
Cherry 2001 (17)	+	+	●
Gray 2002 (18)	+	+	+
Haley 1997 (19)	+	+	+
Kang 2000 (20)¶	+	+	+
Kang 2002 (21)	●	●	+
Kroenke 1998 (8)‖	NS	NS	●
McCauley 2001 (22)	●	●	+
McCauley 2002 (23)	●	●	~/–
Nisenbaum 2000 (24)	+	+	+
Proctor 1998 (25)	NS	+	+
Proctor (27)**	+	+	+
Schumm 2001 (28)	+	●	●
Schumm 2002 (29)	+	●	●
Spencer 2001 (30)	NS	NS	NS
Steele 2000 (1)	●	●	+
Sullivan 2003 (31)	+	●	●
Unwin 1999 (4)	+	+	+
White 2001 (32)	●	+	+
Wolfe 2002 (33)**	+	+	+
SIGN-TEST, 2-sided, spanning AChEi††		$P < 0.0001$	

Statistically significant positive link indicated by "+"; significant negative (protective) link by "–." ●, not tested; ~/–, among 20 reported outcomes, one (lung disease) was significantly lower in Khamisiyah GWV. Additional study information: Cherry *et al.* (17): $P < 0.001$ in whole sample; $P < 0.01$ in both main and validation samples. Exposures most linked to severity were: insect repellent and PB side effects. Only ORs with $P < 0.001$ in whole sample and $P < 0.01$ in validation sample were given. Gray *et al.* (18): PB and pesticides significant in three of three, nerve agent in two of three models. Haley *et al.* (19): No relation of the three syndromes to PB (only to PB side effects, which can occur only with PB use). Kang *et al.* (21): Focused on a "Gulf War specific" factor by factor analysis, present in a much smaller sample. Factor symptoms included blurred vision, loss of balance/dizziness, tremors/shaking, and speech difficulty. McCauley *et al.* (22, 23): 2001 study showed relation between likely low level nerve agent exposure, participation in or witness to Khamisiyah munitions depot demolition (strong exposure evidence), to current health symptoms (outcome elevated in ill GWV). 2002 study found no significant link of measure reflecting weak/uncertain evidence of exposure, being within 50 km of Khamisiyah at demolition, to outcome not generally elevated in ill GWV, hospitalizations or known diagnoses. Additional Reid *et al.* article (34) looked at chronic fatigue syndrome or multiple chemical sensitivities. Pesticides were particularly implicated in MCS, nerve agent exposure (chemical alarm) in both conditions. Spencer *et al.* (30): Study showed strong connection of PB to illness in several models, including dose–response relation. However, study further organized exposures by exposure clusters, and in "final" multivariable model assessing PB through exposure cluster, "exposure to PB was no longer significant."

*Includes relation to PB side effects.

†Includes insecticide, flea collar, personal pesticides in some inquiries (not necessarily exclusive to AChEis).

‡Includes proxy for possible nerve agent exposure like chemical alarms, chemical warfare area, or notified of proximity to Khamisiyah demolition site.

§Bell *et al.* (16): Risk ratios strong but small samples, $n = 9$ to $n = 14$ in comparison groups.

¶Analyzed based on data given.

‖However, the study showed a monotonic increase in reporting of each symptom from neither PB nor pesticide; to either; to both, $P < 0.0001$ sign test.

**Wolfe *et al.* (33) and Proctor *et al.* (26) cite findings for "chemical odor" not specified to be chemical warfare.

††Test contrasts number of significant positive vs. significant negative associations. At most one finding of the same sign (per exposure) is counted for studies with common or strongly overlapping samples (e.g. Proctor, White, and Wolfe studies; Kang studies).

nonsignificant findings are present and virtually no inverse associations.

Results from selected individual studies are shown in Tables 2. For some studies (although not all), not only was each type of AChEi-related exposure significantly linked to illness, but all of the strongest odds ratios for CDC-defined Gulf War illness, among multiple examined exposures, were those relating to AChEis (4), or the only relationships retaining significance on multivariable adjustment were those, and all those, relating to AChEi exposures (24). In fact, a range of other exposures show associations in many studies as well, although these are commonly weaker and/or less consistent. Moreover, compared with AChEi exposures and the broader exposure classes (e.g., pesticides) queried to get at them, the relation of other exposures to multisymptom illness is less consistently retained (perhaps excepting anthrax and multiple vaccines) or is consistently lost (e.g., psychological stressors) in models that adjust for other exposures (17, 24, 25, 30, 33, 35).

Table 2. Relation of AChEi-relevant exposures to illness in GWV

Exposure*	OR (95% C.I.)
PB	2.6 (2.2–3.1)
Chemical or nerve agent attack	2.6 (1.9–3.5)
NBC suits (proxy for PB; sarin; heat)	2.7 (2.3–3.3)
Pesticides (personal pesticides)†	2.2 (1.9–2.6)
Hear chemical alarms (possible proxy for nerve agent exposure)	2.2 (1.9–2.6)

Combat injury showed the strongest relationship but is best understood as a current health problem (vs. exposure) that predicts ongoing future problems (OR 2.9, C.I. 2.1–4.2). Unwin *et al.* (4), 3,284 British GWV.

*Other exposure relationships were also significant. None bore a risk ratio matching these strongest (≥2.2).

†An additional pesticide inquiry, "pesticides in clothes/bedding," showed an OR of 1.9 (95% C.I. 1.6–2.2). Although not among the strongest ORs, it is noted as an AChEi-related exposure for which an odds ratio was provided.

Because exposure questions in epidemiological studies were not specific in identifying chemical class (and servicepersons would not have been likely to be accurate with more specific inquiries), exposure classes that included AChEis, or were proxies for potential AChEi exposures, were used for tabulations for pesticide and chemical agent exposure. [For instance, inquiries about exposures to "pesticides" or "insecticides" are not exclusive to OP and carbamate exposures, although OP and carbamate exposures are known to have been prominent among pesticide exposures in GWV (11, 36) and are considered by the DoD to be the pesticide classes most likely to have a relation to health problems (36), and pesticide exposures were intercorrelated (11, 36).] These factors limit confidence in causal inferences across AChEi classes from epidemiological studies viewed in isolation.

Dose Response. The dose of exposure for nerve agent or pesticide is difficult to characterize. PB pills, however, are given in unit quantities, and personnel can provide an estimate of the number taken (Table 5). In separate studies of U.S. Army GWV (33) and of U.S. female and male GWV, those who reported taking more PB pills had significantly worse reported health state than those citing fewer PB pills (28, 29). In Australian GWV, a significant monotonic worsening occurred across increasing categories of PB use for three physical health outcomes assessed years later (a mental health outcome was not significant) (15).

Of interest, recent evidence suggests that among putatively OP nerve agent-exposed GWV, quantitatively greater estimated OP nerve agent exposure, gauged by DoD plume modeling, is associated with significantly worse neuropsychological function (38) and significantly greater white matter and volumetric loss on quantitative MRI of the brain (39).

Enzymes That Help Detoxify AChEis Differ in Ill Veterans vs. Controls. An objective approach to assessing the probable role of AChEis in illness in GWV that is difficult to ascribe to recall or reporting bias is assessment of functionality of enzymes that detoxify AChEis in veterans who have already declared their illness and exposure state. There is no known way by which subjects can manipulate the

Table 3. Relation of AChEi-relevant exposures to illness in GWV

Significant exposures*	Mild/moderate illness, OR (95% C.I.)	Severe illness, OR (95% C.I.)
PB	1.6 (1.1–2.2) $P = 0.010$	2.9 (1.4–6.1) $P = 0.006$
Insect repellant†	1.7 (1.2–2.3) $P = 0.001$	2.4 (1.3–4.5) $P = 0.006$
Chemical/biological agent exposure‡	2.3 (1.5–3.3) $P < 0.001$	3.5 (1.7–6.9) $P < 0.001$

Multiple polytomous logistic regression adjusted for age, sex, smoking, and current enlisted rank [Nisenbaum *et al*. (24)].

*The sole exposure factors significantly linked to both mild/moderate and severe illness. The sole other significant link was to injuries requiring medical attention: OR 2.1 (95% C.I. 1.1–4.3) severe illness; 1.5 (0.99–2.1) mild/moderate illness.

†The sole pesticide query. Use of different pesticides was correlated (11, 30).

‡Chemical warfare was queried with biological warfare (BW), but no BW exposure occurred.

concentrations and activity levels of these enzymes based on factors like media influence and suggestibility, and there is no evidence to suggest there was differential AChEi exposure in those with different enzyme genotypes and activity levels (because these were not known before the war). For this reason, persons with lesser ability to clear or detoxify AChEis can be considered to have (as a group) an increased exposure "dose" that is not subject to reporting bias. The enzymes known to be involved are paraoxonase (PON) for OPs and butyrylcholinesterase (BChE) for PB.

PON. Each of two major PON types (termed A and B, or Q and R) is superior to the other in hydrolyzing a distinct complement of OPs. Because different veterans were exposed to different subsets of OPs (including various OP pesticides and the OP nerve agent sarin), there is limited *a priori* rationale for supposing that one genetic variant would be more potently associated with illness across all GWV, although veterans with specific known OP exposure might be expected to fare less well if their genetic profile provides for PON alleles less proficient at metabolizing that OP. For this reason, there is no *a priori* rationale for supposing that one or another allele would be associated with greater susceptibility across all GWV. In contrast, concentrations and activity levels of PON are important for clearing all OPs. Therefore, if OP exposure is related to illness in GWV, those with low concentrations and activity levels of PON would be expected to be more susceptible to illness.

Evidence showed that low PON activity levels and concentration are significantly associated with multisymptom health problems in GWV (40, 41) [see supporting information (SI)]. One study also found that ill GWV are more likely to have genetic variants of PON that are less effective at metabolizing certain OPs (including sarin), an effect of borderline significance (40); another study did not find ill GWV to differ significantly from controls in PON genetics (41) (see SI). In a third study, GWV groups were not selected by Gulf War illness criteria, thus limiting inferences (42). Both GWV groups were symptomatic, with low PON1 activity vs. either symptomatic nondeployed Gulf Era and Bosnia deployed groups. The more symptomatic "disabled" GWV group had still lower PON1 activity (trend), less 55LM genotype than less symptomatic GWV ($P = 0.002$ calculated from data provided), and less 192QR genotype than "disabled" persons from the combined other groups ($P < 0.005$, calculated from data provided).

Alterations in PON activity among ill GWV cannot be known to have preceded ill health. In principle, they could reflect contributors to health problems or consequences of exposures among (and selective to) GWV. PON genetics are presumed to precede service; however, directional hypotheses regarding PON genotypes are difficult to generate in GWV because of the range of OP exposures (with their different relationships to PON genotypes). Evidence from outside the GWV setting, in which the OP exposure is known, can strengthen inferences regarding the relation of PON to health in the setting of OP exposures.

BChE. BChE is an enzyme involved in scavenging and inactivating PB (it also has a role in scavenging OPs) (43). Genetic variants less proficient at inactivating PB would be expected to be associated with illness particularly if PB-exposed. Indeed, healthy veterans vs. possibly ill veterans vs. ill veterans in one analysis were successively more likely to have one of the poor-detoxifying BChE variants, such as the K-variant and atypical BChE ($P = 0.02$, χ^2 test of trend) (Table 6) (44). In a small sample of 45 GWV (25 ill, 20 age/sex/education "matched" controls) analyzed for BChE activity (40), those with the lowest quartile of activity had an OR of 2.7 ($P = 0.20$)

Table 4. Relation of AChEi-relevant exposures to illness in GWV

Outcome*	Analysis†	Comparison type‡	Exposure§	Result	95% C.I.	Significance
No. general health symptoms	Negative binomial regression	Ratio of mean no. symptoms (with exposure/without)	PB pills	1.4	1.2–1.5	$P < 0.001$
			Pesticides	1.3	1.2–1.5	$P < 0.001$
			Chemical weapons	1.3	1.2–1.5	$P < 0.001$
Functional impairment in the last 2 weeks	Logistic regression	Odds ratio	PB	1.8	1.2–2.6	$P = 0.004$
			Pesticides	1.5	1.1–2.0	$P = 0.013$
			Chemical weapons	2.3	1.6–3.4	$P < 0.001$
SF-12 PCS	Linear regression	Difference in mean	PB	−2.5	−3.8, −1.2	$P < 0.001$
			Pesticides	−3.4	−4.6, −2.3	$P < 0.001$
			Chemical weapons	−3.7	−5.3, −2.1	$P < 0.001$
SF-12 MCS	Linear regression	Difference in mean	PB	−2.0	−3.6, −1.4	$P = 0.012$
			Pesticides	−3.4	−4.8, −2.1	$P < 0.001$
			Chemical weapons	−4.3	−6.2, −2.3	$P < 0.001$

See Australian GWV study (15).

*Measured outcomes are distinct from "Gulf War illness" definitions but may be expected to be elevated in the setting of Gulf War illness.

†Adjustments: service type, rank, age (<20, 20–24, 25–34, ≥35 years), education, and marital status.

‡Compares exposed group vs nonexposed. Ratio of mean = exposed/unexposed. OR = exposed/unexposed. Difference = exposed − unexposed. For SF-12 PCS and MCS, lower scores are worse.

§Exposures defined as: PB pills (any vs none); pesticides/insecticides; and chemical weapons area.

Table 5. Dose–response: No. PB pills and health in GWV

No. pills	Wolfe (n = 945)* (33) OR (95% C.I.) mild/moderate illness	OR (95% C.I.) severe illness	OR (95% C.I.) multi-variate adjustment	Schumm, female GWV† (n = 113) (28) No. pills	Percent reporting excellent health in most recent year	Schumm, male GWV (n = 474) (29) No. pills	Percent reporting poor to fair health in most recent year	Confined to those citing excellent health in 1990 (n = 221)	Australian GWV (n = 456 with PB pills) (15) No. pills	Mean no. Sx§	PCS score¶	MCS score¶	Percent with functional impairment in last 2 weeks
0	1.0	1.0	1.0	0	51	0	18%	16%	0	11.2	50.8	49.5	15%
1–21	1.9 (1.4–2.7)	2.3 (1.6–3.3)	1.4 (1.0–1.9)	1–10	15	1–2	31%	23%	1–80	14.3	50.0	48.6	14%
>21	2.5 (1.6–3.9)	3.7 (2.4–5.6)	2.1 (1.4–3.1)	>10	6	3–10	38%	32%	81–180	16.9	48.5	44.8	24%
						11–21	44%	41%	>180	16.9	47.8	46.7	31%
						≥22	50%	41%					
						Several blister packs‡	59%	75%					
P	<0.0001‖	<0.0001‖	Not given		<0.0001		<0.0001‖	<0.0001‖		<0.01	<0.001	0.068	<0.001

*n = 1,290 total sample; n = 945 with "full data" from whom ORs were calculated. Multivariate logistic regression model.
†Numbers from Walter Schumm, July 2001. Gamma statistic used (37).
‡ Blister pack had 21 PB pills.
§Among general health symptoms asked.
¶Lower MCS and PCS score implies worse quality of life. Trend calculated in those who took one or more PB pills.
‖Extended Mantel–Haenszel χ^2 test of trend.

Table 6. Correlation of genetic low-metabolizer variants of BChE with self-designated illness status (44)

Described health	N	No. with BChE genetic variants	No. alleles affected
Well	74	22	25
Maybe ill	61	29	30
Sick	91	43	48

P = 0.03 Kendall's Tau rank correlation coefficient (P = 0.012 combining maybe ill and ill) (45).

for illness, although this did not reach significance in this small sample.

In data that were publicly presented, compared with a referent group of persons with neither PB exposure nor sluggish BChE variants, the rate of reported illness was 2.7-fold higher among those citing PB exposure and found to have normal BChE. It was no higher among those citing no PB exposure who were found to have sluggish variants of BChE (thus, the genetic state did not preordain illness irrespective of exposure status). However, among those both citing PB exposure and possessing a low-activity genetic variant of BChE, an ≈40-fold excess risk of reported chronic health problems was found (Antonio Sastre, Research Presentation, Research Advisory Committee on Gulf War Veterans Illnesses, June 16, 2003, http://www1.va.gov/rac-gwvi/docs/Agenda_June2003.doc).

Thus, ill GWV are evidently more likely to have low concentrations and activity levels of enzymes that clear AChEis. Because genetic variants compromising AChEi inactivation are linked to illness, these data provide a particularly compelling character of evidence for a causal link.

Triangulating Evidence: Symptoms in Occupationally OP-Exposed Individuals. If illness were related to AChEi exposure, then similar illness would be expected in previously healthy persons with AChEi exposure in other occupational settings. Individuals with low-level occupational AChEi exposure from agricultural settings report symptoms that mirror those reported by ill GWV. They more commonly report multiple symptoms, and prominent symptoms appear to match those of GWV.

In a postal survey eliciting OP exposure over the prior 10 years, and 10 health symptoms ("yes" or "no") from 175 respondents in an agricultural area (46), 130 (74%) cited OP exposure. Ten times more mean symptoms were reported by the OP-exposed group (2.7 vs. 0.24). Fifty-nine percent of OP-exposed cited at least one of 10 symptoms vs. 13% of unexposed ($P < 0.001$) (46). Among those reporting symptoms, the mean number of symptoms was 4.5 in OP-exposed vs. 1.8 in unexposed. Symptoms queried included fatigue, muscle complaints, and cognitive dysfunction, domains affected in ill GWV. Chemical sensitivity was also reported, a symptom strongly related, among GWV, to pesticide exposure, with an adjusted OR of 12.3 (95% C.I. 5.1–30) (34).

A second study queried 240 individuals who had registered concern about ill health and a possible link to OP exposure to the Organophosphate Information Network; 215 surveys were returned (90% response) (46). One hundred seventy-nine were exposed through sheep dip and 32 through non-sheep dip OPs. Seventy-eight percent of sheep-dip- and 81% of nondip-exposed reported 7–10 symptoms, suggesting similar profiles irrespective of exact OP. Cognitive impairment and reduced exercise tolerance were among the most common symptoms.

A study compared Polish (female) greenhouse workers with OP exposure to similar gardening workers without OP exposure. The OP-exposed group had significant problems with fatigue, muscle, and cognitive dysfunction; additionally, there was evidence of slowed reaction times on neuropsychological tests (47) (see SI).

Triangulating evidence is available regarding enzyme genotypes and activity levels in occupationally OP-exposed British sheep

dippers exposed to the OP diazinon (see SI). Some dippers cite chronic health problems they attribute to dipping. PON genotype and PON diazoxonase activity were examined in 175 dippers who reported health problems they attributed to diazinon and 234 referents of similar age named by the cases, who dipped sheep and were in good reported health (48). Diazinonoxon, the active metabolite of diazinon, is more slowly metabolized by the R than the Q alloenzyme of PON. Dippers with health problems were significantly more likely to have diazoxonase activity below the median of 14.2 μmol/min per ml and significantly more likely to have genetic variants of PON less proficient at metabolizing diazinonoxon, including the R allotype. This is analogous to evidence in ill GWV, in which there are low-activity levels of PON and increased prevalence of low-activity variants of BChE [in ill GWV, the PON variant that is less effective may depend on the OP(s) to which the specific veteran was exposed]. Interestingly, GWV with chronic fatigue show a different bloodflow response to acetylcholine iontophoresis than do civilians with chronic fatigue syndrome, but they match the response of persons with chronic fatigue after OP exposure (49).

Additional triangulating information is emerging from persons with OP AChEi (sarin gas) exposures after terrorist attacks in Japan. Long-term problems with cognition (50, 51), fatigue, and muscle (52), hallmark symptoms of ill GWV, have been reported.

Neither agricultural nor chemical terrorism settings perfectly match the Gulf War experience. Agricultural OP exposures are often low level but repeated over years, longer-term than in GWV. Sarin terrorist exposures were briefer but in higher concentration, often producing acute symptoms. Exposures in GWV varied but may commonly be intermediate between these settings in potency and duration. However, the character of clinical findings reported after exposure in each of these settings appears similar.

Biological Plausibility. In animals, AChEi exposure alters regulation of cholinergic function, which governs domains affected in ill GWV (e.g., muscle function, cognition, sleep). Thus, OPs lead to alteration in densities of cholinergic receptor subtypes (53), including a delayed decrease in M1 muscarinic receptors that occurs in selected brain regions after repeated low-level OP (sarin) exposure (54). Persistent increase in M3 muscarinic receptors arises when exposure cooccurs with heat (54). A persistent change in select nicotinic receptors occurs after low-level repeat OP exposure, coupled with a memory impairment that reverses with nicotine (55). Altered splicing of mRNA for AChE occurs after low-level AChEi exposure (to PB), increasing production of a variant nonsynapse-associated AChE and depressing cholinergic function (56–58). Indeed AChEis induce a multigene transcriptional feedback response that depresses cholinergic action (59). Altered cholinergic regulation is of interest, given the role of cholinergic function in domains of symptom reports in ill GWV. Additionally, low-level AChEi (PB) leads to increased reactive oxygen species and persistent apoptosis of brain cells with muscarinic receptors (60, 61), and low-level OPs persistently alter DNA, protein content, and gene expression in brain of sarin-exposed rats (62, 63).

Biological plausibility is strengthened by this evidence of chronic and delayed consequences to physiological systems from repeated low-level AChEi exposure. Studies that fail to repeat exposures, look for delayed consequences, examine region and system-specific effects, or pick the right outcomes may miss key effects, but as sophistication of studies increases, evidence for persistent and delayed effects of low-level exposure is accruing.

Of note, AChEis could also pertain to the excess of amyotrophic lateral sclerosis (ALS) in GWV, which exceeds already elevated rates among military personnel generally, and is rising (64, 65). Emerging evidence links sporadic ALS to agricultural chemicals (66), PON genotype (67), and perhaps genotype/pesticide interactions (68), compatible with a link between OPs and excess ALS in GWV. Parkinson's disease, a related neurodegenerative condition, has also been linked to pesticides (69, 70) and PON genotype (26, 71), suggesting that monitoring for excess age-adjusted Parkinson's in GWV may be prudent.

Discussion

Evidence, taken together, provides a case for a causal connection of carbamate and OP AChEi exposure to illness in GWV. Epidemiological associations are generally strong. Each of the major types of AChEi exposure that GWV experienced, PB, OP, and carbamate pesticides, and OP nerve agents is linked epidemiologically to illness with remarkable consistency. A dose–response relationship is present, particularly for PB, for which the concept of "dose" is most readily assessed. At the time most of these studies were conducted, there had been no attention to the common mechanism linking these exposures, and in the U.S., little attention was paid to a possible connection of pesticides to illness. [In contrast, depleted uranium had received much greater attention, but the associations to chronic multisymptom illness are more variable and completely absent in some analyses (15).]

Hill's causality criteria, a set of criteria often used for assessment of causality with observational data, are arguably satisfied, including strength and consistency of association, biological plausibility, dose–response relationship (suggested by PON and BChE findings and PB and sarin dose–response relationships), temporality (exposure preceded excess illness: deployed veterans were healthier than nondeployed at the time of deployment, experienced exposures, and are now less healthy), and convergence with other literature (associated with agriculturally exposed persons). The final criterion, of specificity, according to which the exposure should be linked only to the outcome examined, is routinely violated in causal relationships, e.g., alcohol causes not only accidents and liver problems but also neuropathy and cancer.

AChEis used for Alzheimer's disease are typically acridine or piperidine AChEis, not OPs and not typically carbamates, and may or may not have potential for similar sequelae (see SI).

When a link between AChEis and illness was first suggested, a research program was outlined to resolve whether there is a causal link (9). The results of the studies outlined support this suggestion and presumptively implicate a causal connection.

These findings do not imply that all illness in GWV or illness in all GWV is the result of AChEis. However, mounting evidence suggests that AChEi exposure may account for some or perhaps much of the excess illness seen in GWV.

A plausible and substantially supported connection between OP and carbamate AChEi exposure and illness in GWV is important not only for GWV. It has implications for current and future deployments and for homeland defense and may be relevant to a subset of civilians with chronic multisymptom complaints that are currently unexplained.

Methods

Epidemiology. Epidemiological studies with original data assessing the link between carbamate and OP AChEis and symptoms in GWV were identified by using a PubMed search, pairing title words "Gulf War" with "epidemiology" and "acetylcholinesterase inhibitor." Eligible articles and those identified in reference lists were abstracted for tabular presentation of the relation of reported AChEi exposure to illness.

Dose–Response. Articles with original data evaluating a dose–response relationship between AChEis and symptoms (available for PB) were identified and abstracted.

Metabolizing Enzymes That Help Detoxify AChEis Differ in Ill Veterans vs. Controls. Articles examining the link between concentrations, activity levels, or genotypes of enzymes that detoxify carbamate and OP AChEis and multisymptom illness in GWV were identified and abstracted. Although PB is not viewed as a "toxin," for simplicity, we will use the word "detoxify" to refer to actions of enzymes that inactivate either OP or carbamate AChEis. These provide an objective assessment of dose–response data among AChEi-exposed subjects, although

the effect is diluted by inclusion of some subjects who were unexposed to agents that these enzymes help to inactivate.

Triangulating Evidence: Symptoms in Occupationally OP-Exposed Individuals. Articles with original data assessing chronic multisymptom health problems as a function of occupational AChEi exposure and genotypes and activity of AChEi detoxifying enzymes in these groups were identified and abstracted to provide triangulating evidence from outside the Gulf War sphere. Findings were placed in the context of human and animal studies showing biological mechanisms by which low-level AChEi exposure might be linked to chronic health problems.

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1. Steele L (2000) Prevalence and patterns of Gulf War illness in Kansas veterans: association of symptoms with characteristics of person, place, and time of military service. *Am J Epidemiol* 152:992–1002.
2. Fukuda K, *et al.* (1998) Chronic multisymptom illness affecting Air Force veterans of the Gulf War. *J Am Med Assoc* 280:981–988.
3. Iowa Persian Gulf Study Group (1997) Self-reported illness and health status among Gulf War veterans: A population-based study. *J Am Med Assoc* 277:238–245.
4. Unwin C, *et al.* (1999) Health of UK servicemen who served in the Persian Gulf War. *Lancet* 353:169–178.
5. Haley R (1998) Point: bias from the ''healthy-warrior effect'' and unequal follow-up in three government studies of health effects of the Gulf War. *Am J Epidemiol* 148:315–323.
6. White RF (2003) Service in the Gulf War and significant health problems: focus on the central nervous system. *J Psychopathol Behav Assess* 25:77–83.
7. Hotopf M, *et al.* (2003) Gulf War illness–better, worse, or just the same? A cohort study. *Brit Med J* 327:1370.
8. Kroenke K, Koslowe P, Roy M (1998) Symptoms in 18,495 Persian Gulf War veterans. Latency of onset and lack of association with self-reported exposures. *J Occ Environ Med* 40:520–528.
9. Golomb BA (1999) *A Review of the Scientific Literature as It Pertains to Gulf War Illnesses* (RAND, Santa Monica, CA), Vol 2.
10. US Department of Defense (2003) *Environmental Exposure Report. Pesticides. Final Report*. Available at http://www.gao.gov/htext/d04159.html. Accessed May 28, 2007.
11. Fricker RD, *et al.* (2000) *A Review of the Scientific Literature as It Pertains to Gulf War Illnesses* (RAND, Santa Monica, CA), Vol 12.
12. Berardocco D (1997) DoD, CIA release Khamisiyah modeling data. *GulfNEWS* 1:3.
13. US General Accounting Office (2004) Gulf War Illnesses: DOD's Conclusions about US Troops' Exposure Cannot Be Adequately Supported. GAO report number GAO-04–159. Available at http://www.gao.gov/htext/d04159.html. Accessed June 1, 2004.
14. Cecchine G, Golomb B, Hilborne LH, Spektor DM, Anthony CR (2000) *A Review of the Scientific Literature as it Pertains to Gulf War Illnesses* (RAND, Washington, DC), Vol 8.
15. Commonwealth Department of Veterans' Affairs (2003) *Australian Gulf War Veterans' Health Study*. Available at www.dva.gov.au/media/publicat/2003/gulfwarhs/html/executive_summary.htm. Accessed February 20, 2008.
16. Bell IR, Warg-Damiani L, Baldwin CM, Walsh ME, Schwartz GE (1998) Self-reported chemical sensitivity and wartime chemical exposures in Gulf War veterans with and without decreased global health ratings. *Mil Med* 163:725–732.
17. Cherry N, *et al.* (2001) Health and exposures of United Kingdom Gulf War veterans. Part II: The relation of health to exposure. *Occup Environ Med* 58:299–306.
18. Gray GC, Reed RJ, Kaiser KS, Smith TC, Gastanaga VM (2002) Self-reported symptoms and medical conditions among 11,868 Gulf War-era veterans: the Seabee Health Study. *Am J Epidemiol* 155:1033–1044.
19. Haley RW, Kurt TL, Hom J (1997) Is there a Gulf War Syndrome? Searching for syndromes by factor analysis of symptoms. *J Am Med Assoc* 277:215–222.
20. Kang HK, Mahan CM, Lee KY, Magee CA, Murphy FM (2000) Illnesses among United States veterans of the Gulf War: a population-based survey of 30,000 veterans. *J Occup Environ Med* 42:491–501.
21. Kang H, *et al.* (2002) Evidence for a deployment-related Gulf War syndrome by factor analysis. *Arch Environ Health* 57:61–68.
22. McCauley LA, *et al.* (2001) Symptoms of Gulf War veterans possibly exposed to organophosphate chemical warfare agents at Khamisiyah, Iraq. *Int J Occup Environ Health* 7:79–89.
23. McCauley L, Lasarev M, Sticker D, Rischitelli D, Spencer P (2002) Illness experience of Gulf War veterans possibly exposed to chemical warfare agents. *Am J Prev Med* 23:200.
24. Nisenbaum R, Barrett DH, Reyes M, Reeves WC (2000) Deployment stressors and a chronic multisymptom illness among Gulf War veterans. *J Nerv Ment Dis* 188:259–266.
25. Proctor SP, *et al.* (1998) Health status of Persian Gulf War veterans: self-reported symptoms, environmental exposures and the effect of stress. *Int J Epidemiol* 27:1000–1010.
26. Proctor SP, Erickson D, Huang M, Hu H, Wolfe J (1999) Conference on Federally Sponsored Gulf War Veterans' Illnesses Research Program and Abstrat Book (Persian Gulf Veterans Coordinating Board, Arlington, VA), p 34 (abstr).
27. Proctor SP, Harley R, Wolfe J, Heeren T, White RF (2001) Health-related quality of life in Persian Gulf War Veterans. *Mil Med* 166:510–519.
28. Schumm WR, *et al.* (2001) Pyridostigmine bromide and the long-term subjective health status of a sample of female reserve component Gulf War veterans: A brief report. *Psychol Rep* 88:306–308.
29. Schumm WR, *et al.* (2002) Pyridostigmine bromide and the long-term subjective health status of a sample of over 700 male Reserve Component Gulf War era veterans. *Psychol Rep* 90:707–721.
30. Spencer PS, *et al.* (2001) Self-reported exposures and their association with unexplained illness in a population-based case-control study of Gulf War veterans. *J Occup Environ Med* 43:1041–1056.
31. Sullivan K, *et al.* (2003) Cognitive functioning in treatment seeking Gulf War veterans: Pyridostigmine use and PTSD. *J Psychopathol Behav Assess* 25:95–103.
32. White RF, *et al.* (2001) Neuropsychological function in Gulf War veterans: relationships to self-reported toxicant exposures. *Am J Ind Med* 40:42–54.
33. Wolfe J, Proctor SP, Erickson DJ, Hu H (2002) Risk factors for multisymptom illness in US Army veterans of the Gulf War. *J Occup Environ Med* 44:271–281.
34. Reid S, *et al.* (2001) Multiple chemical sensitivity and chronic fatigue syndrome in British Gulf War veterans. *Am J Epidemiol* 153:604–609.
35. Haley RW, Kurt TL (1997) Self-reported exposure to neurotoxic chemical combinations in the Gulf War: A cross-sectional epidemiologic study. *J Am Med Assoc* 277:231–237.
36. Department of Defense (2001) *DoD assessments for events at Al Jubayl remain unchanged*. Available at www.gulflink.osd.mil/news/na_al_jubayl_22feb01.htm. Accessed May 29, 2007.
37. Siegel S, Castellan NJ (1988) *Nonparametric Statistics for the Behavioral Sciences* (McGraw–Hill, New York).
38. Proctor SP, Heaton KJ, Heeren T, White RF (2006) Effects of sarin and cyclosarin exposure during the 1991 Gulf War on neurobehavioral functioning in US army veterans. *Neurotoxicology* 27:931–939.
39. Heaton KJ, *et al.* (2007) Quantitative magnetic resonance brain imaging in US army veterans of the 1991 Gulf War potentially exposed to sarin and cyclosarin. *Neurotoxicology* 28:761–769.
40. Haley RW, Billecke S, La Du BN (1999) Association of low PON1 type Q (type A) arylesterase activity with neurologic symptom complexes in Gulf War veterans. *Toxicol Appl Pharmacol* 157:227–233.
41. Mackness B, Durrington PN, Mackness MI (2000) Low paraoxonase in Persian Gulf War Veterans self-reporting Gulf War Syndrome. *Biochem Biophys Res Commun* 276:729–733.
42. Hotopf M, *et al.* (2003) Paraoxonase in Persian Gulf War veterans. *J Occup Environ Med* 45:668–675.
43. Doctor BP, Saxena A (2005) Bioscavengers for the protection of humans against organophosphate toxicity. *Chem Biol Interact* 157–158:167–171.
44. Lockridge O (1999) *Butyrylcholinesterase genetic variants in persons with Gulf War illness*. Available at www.gulflink.osd.mil/medsearch/GeneticStudies/DoD60.shtml. Accessed February 20, 2008.
45. Wessa P (2008) *Kendall tau rank correlation*. Available at http://wessa.net. Accessed February 20, 2008.
46. Davies DR, Ahmed GM, Freer T (1999) Chronic organophosphate induced neuropsychiatric disorder (COPIND): results of two postal questionnaire surveys. *J Nutral Environ Med* 9:123–134.
47. Bazylewicz-Walczak B, Majczakowa W, Szymczak M (1999) Behavioral effects of occupational exposure to organophosphorous pesticides in female greenhouse planting workers. *Neurotoxicology* 20:819–826.
48. Cherry N, *et al.* (2002) Paraoxonase (PON1) polymorphisms in farmers attributing ill health to sheep dip. *Lancet* 359:763–764.
49. Khan F, Kennedy G, Spence VA, Newton DJ, Belch JJ (2004) Peripheral cholinergic function in humans with chronic fatigue syndrome, Gulf War syndrome and with illness following organophosphate exposure. *Clin Sci (London)* 106:183–189.
50. Miyaki K, *et al.* (2005) Effects of sarin on the nervous system of subway workers seven years after the Tokyo subway sarin attack. *J Occup Health* 47:299–304.
51. Rohlman DS, *et al.* (2007) Neurobehavioral performance of adult and adolescent agricultural workers. *Neurotoxicology* 28:374–380.
52. Abu-Qare AW, Abou-Donia MB (2002) Sarin: health effects, metabolism, and methods of analysis. *Food Chem Toxicol* 40:1327–1333.
53. Henderson RF, *et al.* (2001) Response of F344 rats to inhalation of subclinical levels of sarin: exploring potential causes of Gulf War illness. *Toxicol Ind Health* 17:294–297.
54. Henderson RF, *et al.* (2002) Response of rats to low levels of sarin. *Toxicol Appl Pharmacol* 184:67–76.
55. Stone JD, Terry AV, Jr, Pauly JR, Prendergast MA, Buccafusco JJ (2000) Protractive effects of chronic treatment with an acutely sub-toxic regimen of diisopropylflurophosphate on the expression of cholinergic receptor densities in rats. *Brain Res* 882:9–18.
56. Kaufer D, Friedman A, Seidman S, Soreq H (1998) Acute stress facilitates long-lasting changes in cholinergic gene expression. *Nature* 393:373–377.
57. Lev-Lehman E, *et al.* (2000) Synaptogenesis and myopathy under acetylcholinesterase overexpression. *J Mol Neurosci* 14:93–105.
58. Brenner T, *et al.* (2003) The role of readthrough acetylcholinesterase in the pathophysiology of myasthenia gravis. *FASEB J* 17:214–222.
59. Kaufer D, Friedman A, Seidman S, Soreq H (1999) Anticholinesterases induce multigenic transcriptional feedback response suppressing cholinergic neurotransmission. *Chem Biol Interact* 119–120:349–360.
60. Li L, Shou Y, Borowitz JL, Isom GE (2001) Reactive oxygen species mediate pyridostigmine-induced neuronal apoptosis: involvement of muscarinic and NMDA receptors. *Toxicol Appl Pharmacol* 177:17–25.
61. Li L, Gunasekar PG, Borowitz JL, Isom GE (2000) Muscarinic receptor-mediated pyridostigmine-induced neuronal apoptosis. *Neurotoxicology* 21:541–552.
62. Kassa J, Skopec F, Vachek J (2000) The long-term changes in liver DNA, total protein contents following low level sarin exposure in rats. *Acta Medica (Hradec Kralove)* 43:19–22.
63. Damodaran TV, *et al.* (2006) Gene expression profiles of the rat brain both immediately and 3 months following acute sarin exposure. *Biochem Pharmacol* 71:497–520.
64. Haley RW (2003) Excess incidence of ALS in young Gulf War veterans. *Neurology* 61:750–756.
65. Weisskopf MG, *et al.* (2005) Prospective study of military service and mortality from ALS. *Neurology* 64:32–37.
66. Morahan JM, Pamphlett R (2006) Amyotrophic lateral sclerosis and exposure to environmental toxins: an Australian case-control study. *Neuroepidemiology* 27:130–135.
67. Saeed M, *et al.* (2006) Paraoxonase cluster polymorphisms are associated with sporadic ALS. *Neurology* 67:771–776.
68. Morahan JM, Yu B, Trent RJ, Pamphlett R (2007) A gene-environment study of the paraoxonase 1 gene and pesticides in amyotrophic lateral sclerosis. *Neurotoxicology* 28:532–540.
69. Stephenson J (2000) Exposure to home pesticides linked to Parkinson disease. *J Am Med Assoc* 283:3055–3056.
70. Tuchsen F, Jensen AA (2000) Agricultural work and the risk of Parkinson's disease in Denmark, 1981–1993. *Scand J Work Environ Health* 26:359–362.
71. Benmoyal-Segal L, *et al.* (2005) Acetylcholinesterase/paraoxonase interactions increase the risk of insecticide-induced Parkinson's disease. *FASEB J* 19:452–454.
72. Zintzaras E, Hadjigeorgiou GM (2004) Association of paraoxonase 1 gene polymorphisms with risk of Parkinson's disease: a meta-analysis. *J Hum Genet* 49:474–481.