Persistently Exaggerated Startle Responses in Rats Treated with Pyridostigmine Bromide

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Accepted for publication July 21, 1998

ABSTRACT

Troops in the Persian Gulf War have registered complaints consistent with CNS dysfunction that emerged after returning from the Gulf. A common experience among Persian Gulf War veterans was exposure to pyridostigmine bromide (PB) for prophylaxis against nerve gas exposure. To determine whether PB causes emergent CNS dysfunction, Wistar-Kyoto (WKY) and Sprague-Dawley (SD) rats were given PB for 7 consecutive days in their drinking water. The WKY, but not the SD, rats exhibited a delayed-onset, persistently exaggerated startle response. The WKY rats exhibited exaggerated startle responses that appeared 15 days after the end of PB treatment and were still evident 22 days after the end of treatment. Both the duration and the magnitude of the exaggerated startle responses were related to the dosage of PB. The PB-treated rats exhibited normal short-term and long-term habituation. However, exaggerated startle responses were related to the development of enhanced short-term sensitization. Treating the rats for a second time, 7 weeks after the end of the first PB treatment, induced an exaggerated startle response that appeared sooner and dissipated faster than was evident after the first PB treatment. Inasmuch as the WKY rat has inherently low butyrylcholinesterase activity, a scavenger for PB, these results suggest that prophylactic PB may influence CNS function in individuals with low butyrylcholinesterase activity. Elaboration of the factors that mediate enhanced sensitization in the WKY rat may provide insight into some of the complaints registered by veterans of the Persian Gulf War.

Highly toxic OPs bind irreversibly to AChE, the enzyme responsible for terminating the actions of ACh in nerve terminals and the neuromuscular junction (Taylor, 1990). Threatened use of OPs in the form of nerve gas during the PGW prompted prophylactic treatment with the experimental agent PB (Keeler et al., 1991). In contrast to OPs, the binding of PB to AChE is reversible (Watts and Wilkinson, 1977). The beneficial effects of prophylactic PB are 2-fold: first, PB competitively prevents the irreversible binding of OPs to AChE, and second, dissociation of the reversible binding of PB, after OP exposure has abated, provides free AChE for restoring normal cholinergic function. The recommended dosage of PB for possible nerve gas exposure is 30 mg t.i.d., which corresponds to 20% to 30% inhibition of cholinesterase activity (Keeler et al., 1991).

Also in contrast to OPs, the actions of PB are mainly peripheral. A quaternary carbamate, PB does not readily cross the blood-brain barrier; even at doses that dramatically inhibit blood cholinesterase, PB does not substantially alter brain AChE activity (Murphy et al., 1985). In fact, prophylactic levels of PB have been consistently found not to impair cognitive ability or performance in humans (Cook et al., 1992; Caldwell, 1992; Arad et al., 1992; Wenger and Latzka, 1992; Israeli et al., 1990; Borland et al., 1985), nonhuman primates (Wolthuis et al., 1995; Blick et al., 1993) or rats (Liu, 1991; Shih et al., 1991; Wolthuis and Vanwersch, 1984). Moreover, the effects of PB appear to be transient. Although many troops treated with PB during the PGW reported discomfort from cholinergic overstimulation (frequent urination, flatus, diarrhea, excessive sweating); these mild side effects quickly dissipated upon discontinuation of PB (Caldwell, 1992; Arad et al., 1992).

Since their return from the PGW, many veterans have reported diverse complaints—including fatigue, joint pain, GI problems, sleep disturbances and headaches—with no known medical origin (Haley et al., 1997b; Jamal et al., 1996; Morgan and DaSilva, 1995). Neuropsychological testing indicates that the PGW veterans may also have attentional and memory impairments (Haley et al., 1997a; Morgan and DaSilva, 1995; Jamal et al., 1996). The development of these
persistent signs and treatment with prophylactic PB would seem to be unrelated.

Although a body of research suggests that there is no relationship between prophylactic PB and disturbances in neurological function, recent evidence indicates that prophylactic PB may exert significant CNS activity under certain conditions. Mice exposed to an acute stressor and subsequently treated with prophylactic PB exhibited lower brain AChE levels (Friedman et al., 1996). These researchers proposed two stress-induced mechanisms to account for the significant brain activity of PB: 1) freer access across the blood-brain barrier and 2) more circulating PB through inhibition of BuChE, a scavenger of PB. A role for BuChE is further supported by a report that a soldier with an “atypical” form of BuChE went into cholinergic crisis after exposure to prophylactic PB (Loewenstein-Lichtenstein et al., 1995). In addition, Abou-Donia and colleagues have demonstrated that chemical cocktails containing PB, pesticides and insect repellants can cause greater than expected neurological damage in chickens (Abou-Donia et al., 1996).

In light of these findings, we are reexamining the assumptions regarding CNS activity and prophylactic PB. The evidence cited above suggests that prophylactic PB may significantly influence CNS function under conditions in which BuChE activity has been compromised through exposure to stress or as the product of genetics. The WKY rat, extensively hypertensive rat, has more recently been touted as an animal model of stress sensitivity and depression (Pare, 1994). Second, preliminary data in our laboratory indicate that WKY rats have less BuChE activity than the SD rat (Lim et al., 1989). Second, preliminary data in our laboratory indicate that WKY rats are more sensitive to PB treatment; in a small sample of SD and WKY rats, i.p. administration of PB (2 mg/kg i.p.) induced tremors and more lethal consequences in WKY (6/8) than in SD (2/8) rats (unpublished observations).

To illustrate possible CNS dysfunction consequent to prophylactic levels of PB, we employed the ASR. The essential circuitry of the ASR has been substantially elaborated and comprises the ventral cochlear nucleus, lateral lemniscus nuclei, nucleus reticularis pontis caudalis and spinal motor neurons (Davis, 1989a; Lee et al., 1996). Short-term plasticity and within-session habituation and sensitization depend on the intrinsic circuitry. Long-term plasticity and between-sessions alterations in habituation and sensitization may also involve extrinsic structures, such as the cerebellum (Leaton and Supple, 1986), hippocampus (Koch, 1996), central gray (Fendt et al., 1994) and amygdala (Rosen and Davis, 1990; Davis, 1989b).

Administration of noncompetitive and competitive cholinesterase inhibitors with substantial CNS activity increases startle responsiveness (Overstreet, 1977;Philippines et al., 1996). Increased startle is also observed after treatment with nicotine (Acri et al., 1995; Acri, 1994a; Acri et al., 1991). In contrast, enhanced muscarinic activity after pilocarpine (Overstreet, 1977) or carbachol (Caine et al., 1992; Caine et al., 1991) reduces startle, whereas the muscarinic antagonists atropine and scopolamine lead to increased startle responding (Payne and Anderson, 1967; Williams et al., 1974). Therefore, the increased startle responding after the administration of cholinesterase inhibitors appears to be mediated through nictinic receptors (Philippines et al., 1996).

If extended treatment with PB affects CNS cholinergic neurotransmission, then we would expect PB-treated rats to exhibit exaggerated startle responses. Furthermore, if decreased scavenger activity enhances the ability of PB to affect CNS cholinergic neurotransmission, then we would expect PB treatment to affect startle responding in WKY rats more than in SD rats. Because the CNS signs and symptoms of PGW veterans appeared sometime after their return from the PGW, our experimental plan was to examine startle responding repeatedly after the end of PB treatment.

### Materials and Methods

**Subjects.** Subjects were male SD (n = 32) and WKY (n = 144) rats obtained from Charles River (Wilmington, DE). Rats were housed in single cages in isolation chambers; each chamber housed 16 rats. These chambers are sound-attenuating, with control over light:dark cycles, temperature, air quality and humidity. All rats were allowed 10 days to acclimate to living conditions upon arrival. Light:dark cycles were 12 h:12 h. Rats had access to food and water ad libitum.

**PB treatment.** PB (Sigma Chemical Co., St. Louis, MO) was dissolved in tap water. Our interest was developing a treatment protocol that noninvasively delivered PB and produced approximately 20% inhibition of BuChE activity (corresponding roughly to the treatment protocol used in the PGW). Rats were administered 0.009, 0.018 or 0.045 mg/l PB in the drinking water for 7 consecutive days. The first day of PB treatment was considered day 1, with subsequent time references relative to this point. For example, the day after PB treatment was day 8. Over the 7-day treatment period, rats given 0.0, 0.009, 0.018 and 0.045 mg/l PB drank 43 ± 2.3, 41 ± 1.1, 42 ± 1.6 and 44 ± 1.5 ml/day, respectively. The dose of PB ingested over the treatment period was computed to be 1.3 ± 0.1, 2.6 ± 0.1 and 7.2 ± 0.4 mg/kg b.wt./day, respectively, for the PB-treated rats. The different concentrations of PB did not affect drinking behavior.

However, substantial differences were evident between the rat strains. As can be seen in table 1, treatment with PB did not affect the consumption of drinking water or food intake during treatment. However, the strains differed substantially in body size at the same age. The differences in body size resulted in the SD rats ingesting more PB than the WKY rats.

**TABLE 1**

Effects of the PB treatment protocol in SD and WKY rats

<table>
<thead>
<tr>
<th></th>
<th>SD-CON n = 8</th>
<th>SD-PB n = 8</th>
<th>WKY-CON n = 12</th>
<th>WKY-PB n = 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>402 ± 9</td>
<td>394 ± 6</td>
<td>297 ± 2</td>
<td>297 ± 2</td>
</tr>
<tr>
<td>Growth rate (g/d)</td>
<td>6.1 ± .4</td>
<td>6.0 ± .5</td>
<td>1.8 ± 2</td>
<td>1.5 ± 1</td>
</tr>
<tr>
<td>Water intake (ml/d)</td>
<td>61 ± 9</td>
<td>62 ± 9</td>
<td>40 ± 2</td>
<td>40 ± 2</td>
</tr>
<tr>
<td>Base-line BuChE activity (U/ml)</td>
<td>389 ± 48</td>
<td>367 ± 27</td>
<td>200 ± 7</td>
<td>204 ± 4</td>
</tr>
<tr>
<td>PB dose (mg PB/kg BW/d)</td>
<td>2.56 ± .08</td>
<td>2.56 ± .08</td>
<td>2.28 ± .06</td>
<td>2.28 ± .06</td>
</tr>
</tbody>
</table>

* All entries are expressed as the mean ± S.E.M. The SD rats (n = 8 for the PB and CON groups) differed significantly from the WKY rats (n = 12 for the PB and CON groups) in all the variables; P < .05.
Plasma BuChE activity. Blood samples were collected at 0900 in heparinized hematocrit tubes and centrifuged to separate plasma; then the plasma was stored frozen (−80°C) until assayed. The Sigma reagent set (#422-10), which is based on the colorimetric method of Ellman (Ellman et al., 1961), was used for the determination of BuChE activity. The substrate for hydrolysis was propionylthiocholine, and optical density was read at 405 nm. Multiple determinations of several samples yielded $r^2 = 0.92$, the slope was not different from 1.0, and the intercept was not different from 0.

Startle testing. The startle apparatus (rat holders, platforms, white noise generators and interface) was obtained from Coulbourne Instruments (Langhorne, PA). The software package (Viewdac) used to control stimulus presentation and signal recording and the A/D board (DAS 1600) were obtained from Keithley-Metrabyte (Tauton, MA). The acoustic stimuli were bursts of white noise (100 ms, 5-ms rise/fall time) of 82, 92 or 102 dB. To control for circadian variations in startle responding, rats were run in pairs, with a representative from the PB treatment and control groups run at the same time each measurement day.

Two startle protocols were employed. In the first, rats were exposed to white noise stimuli of three intensities (82, 92 and 102 dB; 5-ms rise/fall). Rats were exposed to eight stimuli of each intensity. A single block-random order was used for all startle test sessions. The interstimulus interval (ISI) ranged from 25 to 35 s. The startle data for each session were reduced by obtaining the mean values for each rat at each stimulus intensity level.

For the second protocol, rats were repeatedly exposed to a single intensity of white noise stimuli (102 dB; 5-ms rise/fall). Rats will typically emit a startle response on all 60 trials. The data were reduced by forming 10 trial blocks composed of the mean values for six consecutive trials. This protocol is amenable to characterizations of within-session patterns in the magnitude of startle responding—that is, habituation and sensitization. Short-term or within-session habituation was defined as decreased startle magnitudes occurring from the first trial block over the next four trial blocks. For short-term sensitization, a sensitization index (SI) was computed for each startle session as the sum of the magnitudes of startle responses over the last five trial blocks that were greater than that in the initial trial block. Long-term habituation (or sensitization) was defined as significant decreases (or increases) in startle response magnitudes from the first trial block of the pretreatment startle session to the first trial block in post-treatment startle sessions. The ISI was 15–25 s.

Signal processing. Substantial differences in body size between these two rat strains necessitated that A/D activity (sampled at 1000 Hz) be divided by body weight. For each stimulus presentation, a response threshold was computed as the average rectified activity 200 ms before stimulus onset plus six times the standard deviation of that rectified activity. Response amplitudes, the maximal rectified activity within 200 ms after stimulus onset, were recorded only when poststimulus activity exceeded the response threshold.

Statistics. Data were analyzed with $t$ tests for two-group comparisons and with split-plot ANOVA models for comparison of groups with repeated measures. Specific $a$ priori comparisons were accomplished with Dunnett’s and Dunn’s tests. Significance levels were set at $P < .05$ for all post-hoc comparisons.

Results

PB treatment. As can be seen in table 1, the rats from the two strains differed in their initial body weight, as well as in their growth rate and water consumption during PB treatment. Moreover, the SD-PB rats (2.56 ± 0.08 mg/kg b.wt./day) ingested a slightly, but significantly, higher dose of PB than the WKY-PB (2.28 ± 0.10 mg/kg b.wt./day) rats, $t(18) = 2.0$, $P < .05$.

Plasma BuChE activity. Base-line blood samples were obtained 1 day before PB treatment. The BuChE activity in WKY rats (235 ± 13) was significantly lower than that in SD rats (321 ± 32), $P < .05$. These samples were compared with samples obtained on days 3, 6 and 10 (3 days after the end of
PB treatment). Inhibition of BuChE activity was reduced by about 20% by the 7-day PB treatment (0.018 mg/l) protocol in both SD and WKY rats (fig. 1). In addition, plasma BuChE activity recovered to pretreatment levels by 3 days after the end of treatment. This was confirmed by a 2-way ANOVA. Significant main effects of Drug, $F_{(2,407)} = 8.0$, and Measurement Day, $F_{(2,407)} = 23.4$, were all significant. These were modified by the PB Dose x Measurement Day interaction, $F_{(2,407)} = 4.3$, PB Dose x Measurement Day, $F_{(6,407)} = 8.0$, and Stimulus Intensity x Measurement Day, $F_{(4,407)} = 4.0$, interactions; all $P < .05$.

**ASRs.** We assessed startle responding in SD and WKY rats using the multiple-intensity protocol on the last day of PB treatment (day 7) and weekly for the next 2 weeks (days 15 and 22), a total of three startle measurement sessions. An exaggerated startle response developed in WKY rats treated with PB (fig. 2). The WKY rats treated with PB exhibited an exaggerated startle response on day 21, 14 days after the end of PB treatment. The magnitudes of the startle response were analyzed with a 2 x 2 x 3 (Strain x Drug x Measurement Day) mixed ANOVA. The main effects of Drug, $F_{(2,208)} = 83.6$, and Measurement Day, $F_{(2,407)} = 23.4$, were all significant. These were modified by the PB Dose x Measurement Day interaction, $F_{(2,407)} = 3.9$, were qualified by the significant Drug x Measurement Day interaction, $F_{(2,52)} = 9.57$; all $P < .05$.

A dose-response relationship was revealed in WKY rats treated with either 1.3, 2.6 or 7.2 mg PB/kg/day (see fig. 3). Exaggerated startle responses were evident in all PB-treated rats on day 15, 8 days after the end of PB treatment. By day 22, the startle responses of the rats given the lowest dose of PB had recovered to the level of the CON rats. Exaggerated startle responses were still evident in the rats given the two higher doses of PB. Moreover, the magnitudes of the startle responses were dose-related. Rats ingesting 7.2 mg PB/kg/day exhibited significantly higher startle responses than rats given 2.6 mg PB/kg/day, and the startle responses of both of these groups were greater than those of rats given 1.3 mg PB/kg/day and CON rats. These impressions were confirmed with a 4 x 3 x 3 (Drug Treatment x Stimulus Intensity x Measurement Day) mixed ANOVA. The main effects of Drug Treatment, $F_{(3,47)} = 4.0$, Stimulus Intensity, $F_{(2,407)} = 83.6$, and Measurement Day, $F_{(2,407)} = 23.4$, were all significant. These were modified by the PB Dose x Stimulus Intensity, $F_{(6,407)} = 4.3$, PB Dose x Measurement Day, $F_{(6,407)} = 8.0$, and Stimulus Intensity x Measurement Day, $F_{(4,407)} = 4.0$, interactions; all $P < .05$.

Further, we sought to determine whether the exaggerated startle responses of WKY rats were the result of changes in habituation or sensitization. Using a single-intensity startle protocol, we exposed WKY rats to 0.018 mg/ml PB for 7 consecutive days. These rats exhibited exaggerated startle responses on days 22 and 28—that is, 15 and 22 days after the end of PB treatment, respectively. The exaggerated startle responses were not the result of decreases in short-term (within-session) habituation. Moreover, there was no evidence that PB treatment affected either long-term (between-sessions) habituation or sensitization. However, the rats treated with PB exhibited short-term startle sensitization 15 and 22 days after the end of PB treatment (see fig. 4). Short-term sensitization was analyzed in a 2 x 6 (PB Treatment x Measurement Day) mixed ANOVA. The PB Treatment x Measurement Day interaction was significant, $F_{(5,130)} = 2.64$, $P = .02$.

In addition, a second PB treatment was begun on day 56, 7 weeks after the end of the first PB treatment. For this second treatment, four groups were formed: rats previous treated with PB and given a second treatment (PB-PB group), rats...
given PB treatment for the first time beginning on day 56 (CON-PB group), rats previously treated with PB and then given tap water (PB-CON group) and rats given only tap water in both phases (CON-CON group). Startle responses of the PB-PB rats were greater than the responses of all the other groups on day 77, 8 days after the end of the second PB treatment (fig. 5). However, startle responses were normal in rats treated with PB for the first time on day 63. Therefore, a second treatment with PB did not produce more pronounced neurobehavioral disturbances in WKY rats. Moreover, the second 7-day PB treatment produced about the same degree of plasma BuChE inhibition as a single 7-day PB treatment (data not shown).

Analgesia. To determine whether PB treatment persistently altered pain responses, hot-plate latencies were measured in SD and WKY rats on day 11 (4 days after the end of PB treatment). Pain tolerance was defined as the latency to paw-lick in SD and WKY rats. Although there was a pronounced difference in pain sensitivity between the SD and WKY rats, treatment with PB did not persistently affect pain tolerance (see Figure 6A). A 2 × 2 (Strain × Drug Treatment) ANOVA indicated a main effect only of Strain, F(1,26) = 5.2, P < .05. The paw-lick latencies of WKY rats (4.4 ± 0.3 s) were shorter than those of the SD rats (6.0 ± 0.6 s).

Activity. To determine whether PB treatment persistently altered reactivity to a novel environment, we measured open-field activity in SD and WKY rats on day 11 (4 days after the end of PB treatment). Open-field activity was defined as the number of sections entered over a 2-min test period. Like pain sensitivity, open-field activity differed substantially with strain. Treatment with PB did not persistently affect open-field activity (fig. 6B). The WKY rats (3.6 ± 1.0 sections entered) were less active in the open field than the SD rats (19.9 ± 3.9 sections entered). A 2 × 2 (Strain × Drug Treatment) ANOVA indicated a significant main effect only of Strain, F(1,26) = 15.3, P > .001.

Discussion

The military has endorsed a treatment protocol for possible nerve gas exposure that includes prophylactic PB at 30 mg/kg t.i.d. for 7 consecutive days. This treatment protocol is designed to produce 20% to 30% inhibition of plasma BuChE activity, a convenient index of AChE activity. We have attempted to devise a treatment regimen in rats that mimics some of the features of the military protocol. Delivery of PB at 0.018 mg/l in the drinking water produced 15% to 20% inhibition of BuChE activity in rats. Blood samples were obtained 2 h after the onset of the light phase. Inasmuch as rats drink approximately 80% of their water during the dark phase, it is likely that the levels of inhibition that we obtained represent steady-state rather than peak values. Moreover, rats exhibited some of the mild signs of cholinergic overstimulation, such as excessive lacrimation and diarrhea, signs also exhibited by troops during the PGW (Cook et al., 1992). However, drinking-related behaviors appeared to be unaffected; in the presence of these PB-induced signs of cholinergic overstimulation, total daily water consumption and growth rate did not differ from those of control rats. Therefore, treatment with PB in the drinking water of rats appears to be an adequate model of the treatment protocol employed by the military during the threat of nerve gas exposure.

Our primary goal was to assess the long-term or persistent effects of prophylactic PB on the acoustic startle response. Treatment with prophylactic PB for 7 consecutive days did not alter the acoustic startle responding of SD rats. In con-

![Fig. 3. Effect of PB dosage on the ASR of WKY rats. WKY rats ingested 1.3, 2.6 or 7.2 mg PB/kg/day for 7 days or served as nontreated controls (n = 14 for all groups). Startle measurement, using the multiple-stimulus intensity protocol, began 1 day after the end of the 7-day PB-treatment protocol. Comparisons with CON values are indicated by *. Comparisons with initial startle data (day 8) are indicated by &. & indicates comparisons between the PB-7.2 and PB-2.6 groups. For all, P < .05.](attachment:image.png)
The WKY rats treated with PB exhibited exaggerated startle responses. The exaggerated startle responses had a delayed appearance in that they emerged 15 days after the end of PB treatment in WKY rats. A dose-response relationship was also indicated. The WKY rats treated with PB at 1.3, 2.6 and 7.2 mg/kg exhibited exaggerated startle responses on day 15, 8 days after the end of PB treatment. By day 22, the startle responses of the rats treated with 1.3 mg/kg recovered to control levels. However, exaggerated startle responses were still evident in rats given 2.3 and 7.2 mg PB/kg, the greatest responses being exhibited by rats given 7.2 mg PB/kg. Therefore, the development and degree and startle abnormality in WKY rats appear to be related to the dose of PB ingested.

Enhanced short-term sensitization so long after the end of PB treatment suggests that the exaggerated startle responses exhibited by WKY rats were the result of PB-induced alterations in CNS activity. Such an effect of prophylactic PB would be unexpected, given that the actions of prophylactic PB are considered to be wholly peripheral. Friedman and colleagues have suggested that the influence of PB on CNS cholinergic activity may be inversely related to plasma BuChE activity; BuChE is a scavenger for PB. Lower BuChE activity, whether as a result of an inherited deficit or through stress-induced reductions, may give circulating PB an increased opportunity to cross the blood-brain barrier and thereby affect central AChE activity. Under Friedman’s formulation, the lower plasma BuChE activity of WKY rats, 40% to 50% less than that of SD rats, may account for our functional evidence of altered CNS activity after prophylactic PB in WKY rats, but not SD rats. Thus the initial state of BuChE activity may be an important determinant of CNS effects after prophylactic PB.

On the other hand, these strains differ markedly in their response to mild stressors (Paré, 1994; Paré and Redei, 1993;
Fig. 6. Persistent effects of PB treatment on pain sensitivity and open-field activity. Pain sensitivity (panel A) and open-field activity (panel B) were measured on day 11, 4 days after the end of PB treatment. These rats were same as those described in figure 2. Only strain differences were noted for both behavioral measures.

Fig. 5. Startle response magnitude of WKY rats after a second PB treatment. Of rats given an initial PB treatment in figure 4, rats either were given a second exposure to the PB treatment protocol (PB-PB rats, n = 6) or served as nontreated controls (PB-CON, n = 6). Of nontreated rats, rats either were given an initial PB treatment on day 56 (CON-PB, n = 6) or served as nontreated controls (CON-CON, n = 7). Comparisons with CON values are indicated by *.
Paré, 1992; Paré, 1989). In a result consistent with this work, the WKY rats exhibited a greater sensitivity to pain and greater reactivity to a novel environment than did SD rats. For the WKY rats, treatment with PB may represent a form of interoceptive stress. The development of exaggerated startle responses in the WKY rats may be an overt response to interoceptive stress, analogous to somatization.

Even allowing for the entry of PB into the CNS, the mechanism by which startle responding may be altered so long after the end of PB treatment is unclear. Administration of phystostigmine, a centrally active AChE inhibitor, increases startle responding in guinea pigs (Philippens et al., 1986). This increased startle responding was potentiated by scopolamine, a muscarinic antagonist. Exaggerated startle responses were evident 30 min after injection, but normal startle responding was observed 24 h after injection. In that scopolamine will increase nicotinic neurotransmission (Consolo et al., 1991), the exaggerated startle responses may have been the result of enhanced nicotinic activity (Philippens et al., 1996). Acri and colleagues have shown that enhanced nicotinic activity results in exaggerated startle responding (Acri et al., 1991; Acri, 1994a; Acri et al., 1995; Acri, 1994b). Moreover, rats administered nicotine for 10 days exhibit exaggerated startle responses during withdrawal (Acri et al., 1991). The exaggerated startle responses were not evident 1 to 3 days after the cessation of nicotine administration but appeared 5 to 7 days after the cessation of treatment. Therefore, exaggerated startle responding may be mediated through nicotinic receptor hypersensitivity during withdrawal. However, other CNS mechanisms are possible. For example, the exaggerated startle responses may reflect alterations in circadian rhythms. Administration of diisopropyl phosphorofluoridate (DFP), an OP inhibitor of AChE, alters circadian rhythms (Raslear et al., 1986), and startle responses vary as a function of time of day (Chabot and Taylor, 1992; Chabot and Taylor, 1992a; Frankland and Ralph, 1995; Horlington, 1970).

How and whether these results generalize to explain the unexplained illness of PGW veterans, particularly the signs of CNS dysfunction, is uncertain. Although the number of troops with impaired BuChE activity before PB treatment is unknown, an alteration in cholinergic state may not be rare. As mentioned, lower BuChE activity has been observed after exposure to stress or as a result of genetics. Our findings suggest that such individuals may have been at greater risk of persistent CNS dysfunction after pyrophylactic PB treatment during the Gulf War.

References


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