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Potentiation of Pyridostigmine Bromide Toxicity in Mice by Selected Adrenergic Agents and Caffeine

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ABSTRACT. Pyridostigmine bromide (PB) is a reversible cholinesterase inhibitor used routinely in the treatment of myasthenia gravis and recently by the US Army as a prophylactic agent against potential nerve gas attack in the Persian Gulf War. Pyridostigmine has been implicated as one of several possible causative factors associated with Persian Gulf illnesses. To investigate toxic interactions between PB and other drugs, male ICR mice received contralateral ip injections of either a selected adrenergic drug or caffeine, followed 15 min later by PB. Representative isobolograms plotted for each drug interaction illustrate that a β-adrenoceptor agonist (isoproterenol), selective β_2-adrenoceptor agonists (salbutamol, terbutaline), α_1- and α_2-adrenoceptor antagonists (yohimbine, phentolamine, prazosin), as well as the stimulant caffeine, strongly potentiate the lethal effect of PB. Agents with agonist activity at both α- and β-adrenoceptors (epinephrine, norepinephrine) additively increase PB-induced lethality. The potentiation of toxicity between PB and these agents was counteracted by pretreatment with atropine and atropine methyl nitrate. An α_2-adrenoceptor agonist (clonidine) and β-adrenoceptor antagonists (propranolol, nadolol, acebutolol) did not increase PB-induced lethalities. These data demonstrate a toxic synergism between PB, several commonly used classes of adrenergic agents and caffeine when exposure occurs in different combinations. Future studies into the mechanism(s) of these interactions may bring into question the usage of PB as a protective agent in combat conditions as well as delineate any possible contributions of the drug to Persian Gulf illnesses.

Pyridostigmine bromide (PB) is a quaternary dimethyl carbamate that has been used historically at relatively high doses (200-1400 mg/d) treat myasthenia gravis, a neuromuscular disorder characterized by skeletal muscle weakness and fatigability (1). Since 1986, PB has been recommended by the US Army for use as a prophylactic agent against organophosphate (OP) nerve gases (2). Organophosphates bind irreversibly to acetylcholinesterase (AChE) in the central (CNS) and peripheral (PNS) nervous system to prevent hydrolysis of acetylcholine (ACh) at sites of cholinergic transmission. As a result, ACh accumulates at cholinergic receptor sites, producing excessive parasympathetic stimulation leading ultimately to muscle paralysis and death. In contrast, prophylactic doses of PB (30 mg, po, tid) reversibly inhibit 30-40% of AChE activity in the PNS to protect it from permanent inactivation by OP chemical warfare agents. Spontaneous dissociation over time restores an adequate level of AChE activity to maintain life, providing that atropine and oxime treatments are also administered at the time of exposure (3). It is assumed that PB does not cross the blood-brain barrier to enter the CNS, since it is a positively charged agent and therefore should not interfere with cognitive function (3).

The first usage of PB in a combat situation occurred during the Persian Gulf War. Between August 1990 and April 1991, approximately 700,000 US troops were deployed to the Persian Gulf where some were exposed simultaneously to a variety of potentially toxic insults, including chemical warfare agents, toxic fumes, pesticides, infectious diseases, multiple immunizations, chemoprophylactic agents and psychological stress (4). Since returning from the war, an estimated 5,000-80,000 soldiers have complained of a persistent array of inexplicable symptoms ranging from headache, loss of memory, fatigue, muscle and joint pain, and ataxia, to skin rash, respiratory difficulties and gastrointestinal disturbances (5). Pyridostigmine has been implicated as a possible causative agent linked to these illnesses based on reports of acute side effects which were experienced by a number of soldiers during the Gulf War (4,6), the questionable compliance of troops to the recommended dosing regimens (7), lack of knowledge in regard to drug interactions which might increase the acute or chronic toxicity of the drug (6), and the unknown response of the human body to the drug under extreme conditions of stress comparable to those encountered in war (4,6).

Enhancement of activity within the sympathetic (adrenergic) nervous system is one of many responses to psychological stress, in addition to other changes in CNS function, behavioral alterations, and disturbances in the immune, cardiovascular and gastrointestinal systems (8). Friedman et al (9) showed that stress-induced alterations in the blood-brain barrier may allow PB to act centrally, as demonstrated by decreased brain AChE activity and increased brain levels of c-fos oncogene and AChE mRNAs following peripheral administration of PB under conditions of stress. Recent investigations have linked the combined exposure to PB and the insect repellent N,N-diethyl-m-toluamide (DEET) to increased lethality in rats (10), chickens (11), and mice (12). In studies of interactions between hydrolytic enzyme inhibitors and DEET in German cockroaches, Moss (13) proposed that DEET may have inherent adrenergic activity that could play a role in its toxic interaction with PB.

Based on the premise that activation of the sympathetic nervous system could be involved in an adverse response to PB, we chose to investigate combinations of PB and selected adrenergic agents to probe the possibility of an acute interaction between an enhanced cholinergic state and altered adrenergic activity. Several of these agents are commonly prescribed for the treatment of asthma and for regulation of blood pressure (14). Caffeine was also included in this study because of its prevalence in common stimulatory beverages and its ability to increase levels of circulating catecholamines (15).

MATERIALS AND METHODS

Chemicals

Pyridostigmine bromide, (±)-epinephrine hydrochloride, (±)-arterenol bitartrate salt (norepinephrine), (±)-isoproterenol hydrochloride, salbutamol, terbutaline hemisulfate, yohimbine hydrochloride, phentolamine hydrochloride, prazosin hydrochloride, caffeine, clonidine hydrochloride, DL-propranolol hydrochloride, nadolol, acebutolol hydrochloride, atropine sulfate and atropine methyl nitrate were purchased from Sigma Chemicals (St Louis, MO).

Animals

Male ICR mice (20-24 g) were purchased from Harlan Sprague Dawley Inc (Indianapolis, IN) and housed in the central animal facility which was maintained at 22 ± 1 C with a 12-h light-dark cycle. Tap water and laboratory chow were provided ad libitum. Mice were randomly assigned to treatment groups, housed in plastic cages with 6-10 animals/cage, and allowed to acclimate to the animal facility environment for 48 h before dosing.

Drug Treatment

All drugs were dissolved in distilled water immediately prior to each experiment. Individual drugs were administered ip at varying doses in a volume of 10 mL/kg body weight to groups of 6-10 mice, and the number of deaths were recorded after 24 h. Fixed doses of adrenergic drugs and caffeine for drug interaction studies were selected at referenced doses (16,17) and demonstrated to cause no adverse effect: epinephrine, 5 or 10 mg/kg; norepinephrine, 7.3 or 10 mg/kg; isoproterenol, 3 mg/kg; salbutamol, 0.4 or 0.8 mg/kg; terbutaline, 5 mg/kg; phentolamine, 1 mg/kg; prazosin, 2 mg/kg; yohimbine, 1

mg/kg; caffeine, 5 mg/kg; clonidine, 1 mg/kg; propranolol, 1.5 or 3 mg/kg; nadolol, 1 or 5 mg/kg; and acebutolol, 1 or 5 mg/kg. Single ip injections of these agents were administered at the above doses to groups of 6-10 animals, followed 15 min later by a single contralateral ip injection of PB (1, 2 or 3 mg/kg). Mice were returned to their cages and observed continuously for 1 h after dosing. Number of deaths was recorded after 24 h.

In a separate study, groups of 6-10 animals were pretreated with a muscarinic antagonist prior to several of the above drug combinations. Mice were given simultaneous contralateral ip injections of either atropine (10 mg/kg) or atropine methyl nitrate (5.4 mg/kg) and an adrenergic agent (epinephrine, 5 mg/kg; norepinephrine, 7.3 mg/kg; salbutamol, 0.8 mg/kg; yohimbine, 1 mg/kg) or caffeine (5 or 10 mg/kg), followed 15 min later by PB (3 mg/kg), and lethality was recorded over a 24 h period.

Data Analysis

LD_{50} values and 95% confidence limits (CL) for individual drugs and for PB in the presence of a constant dose of each adrenergic drug or caffeine were calculated using a computerized version of the Litchfield Wilcoxon analysis (18). Isobolograms were then plotted to establish whether combined drug effects were greater, equal or less than would be expected based on the individual activity of the drugs and the theory of dose additivity, as discussed by Gessner (19). Briefly, an isobologram is a graphic representation of equieffective doses of 2 drugs, using rectangular coordinates, such that the coordinate plane is defined by the dose axes of each drug. An isobol is a contour line, or line of simple addition, where all points represent equieffective quantities of the 2 drugs. LD_{50}s and 95% CL for co-administered drugs can then be plotted on the same graph, and drug interactions can be evaluated based on where the points fall in relation to the line of additivity. If a point falls below the line of additivity, without overlapping CLs, the interaction is considered to be supra-additive, indicating potentiation of effects.

Table 1. LD50s with 95% CL for individual drugs at 24 hrs.

Drug	24 hr LD50 (mg/kg)	Lower 95% CL	Upper 95% CL
caffeine	251.3	234.3	269.4
epinephrine	12.8	8.5	19.1
isoproterenol	255.3	35.0	1860.5
norepinephrine	30.9	23.7	40.2
phentolamine	104.3	89.2	122.0
PB	4.3	3.9	4.9
salbutamol	166.5	155.0	178.9
terbutaline	186.3	168.9	205.4
yohimbine	42.1	7.5	237.4

RESULTS

The 24 h LD_{50}s and 95% CL for individual drugs are reported in Table 1. The LD_{50} of prazosin was not determined due to solubility difficulties at such high doses. Instead, an LD_{50} value of 60 mg/kg with 95% CL between 47-77 mg/kg was assumed for prazosin based on a report by Noguchi et al (20) for ip dosing in male ICR mice. The above data were used in conjunction with LD_{50} data derived from the binary mixtures of each drug with PB to plot isobolograms.

In the drug interaction studies, control animals that received the selected doses of each adrenergic agent or caffeine alone did not exhibit adverse effects, except for epinephrine and norepinephrine, both of which

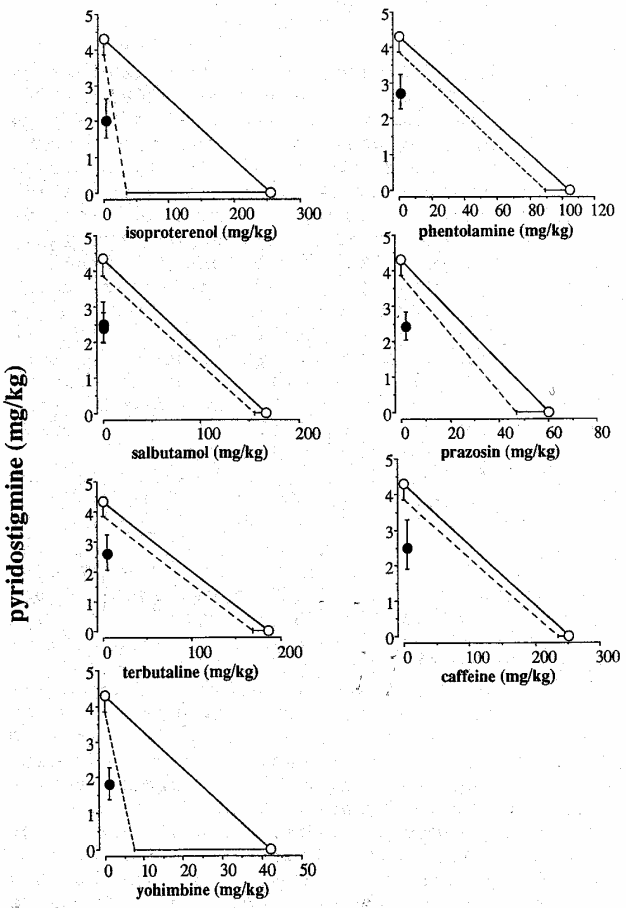

Figure 1. Isobolograms illustrating the combined lethal effects (LD_{50}) of PB with fixed doses of isoproterenol (3 mg/kg), salbutamol (0.4 or 0.8 mg/kg), terbutaline (5 mg/kg), yohimbine (1 mg/kg), phentolamine (1 mg/kg), prazosin (2 mg/kg) or caffeine (5 mg/kg). The open circle at the upper left of each isobologram represents the LD_{50} with 95% confidence limits (CL) of PB alone, while the corresponding open circle at the lower right depicts the LD_{50} with 95% CL for the second drug alone. Solid and dashed negative slope diagonals represent the locus of expected LD_{50} points for combinations of the 2 agents, and the lower 95% CL of this line, respectively, given dose additivity. Solid circles represent the LD_{50} points with 95% CL for binary combinations of the drugs in mice.

Table 2. LD50s with 95% CL for PB following administration of adrenergic drugs at fixed doses.

Adrenergic drug (mg/kg) + PB	24 hr LD50 (mg/kg)	Lower 95% CL	Upper 95% CL
caffeine (5)	2.5	1.9	3.3
epinephrine (5)	2.6	2.2	3.1
epinephrine (10)	0.9	0.3	2.3
isoproterenol (3)	2.0	1.5	2.6
norepinephrine (7.3)	2.2	1.8	2.9
norepinephrine (10)	2.2	1.3	3.6
phentolamine (1)	2.7	2.3	3.2
prazosin (2)	2.4	2.0	2.8
salbutamol (0.4)	2.5	2.0	3.1
salbutamol (0.8)	2.4	2.0	2.8
terbutaline (5)	2.6	2.1	3.3
yohimbine (1)	1.8	1.4	2.3

caused slight tremor, increased respiration and decreased activity in the animals. Pyridostigmine administered alone at 1 mg/kg did not produce any adverse reactions; however increasing the dose to 2 mg/kg produced skeletal muscle tremor and inactivity, while 3 mg/kg caused tremor, lacrymation, salivation and respiratory depression resulting in approximately 12.9 ± 3.8 (SE) % lethality, with convulsions immediately preceding death. Pretreatment with selected doses of the β-adrenoceptor agonist isoproterenol, selective β_2-adrenoceptor agonists salbutamol and terbutaline, α_1- and α_2-adrenoceptor antagonist phentolamine, selective α_1-adrenoceptor antagonist prazosin, selective α_2-adrenoceptor antagonist yohimbine, and the stimulant caffeine significantly increased lethality when combined with PB at the doses tested, as shown in Fig 1. The observed LD_{50}s and 95% CL (Table 2) for each drug combination did not overlap the 95% CL for the line of dose additivity, providing clear evidence of potentiation of the drug interaction (19), even in the cases of yohimbine and isoproterenol where large CL were noted. The α- and β-adrenoceptor agonists epinephrine or norepinephrine, combined with PB, produced only an additive increase in lethality as illustrated in Fig 2. The α_2-adrenoceptor agonist clonidine and β-adrenoceptor antagonists propranolol, nadolol and acebutolol did not increase PB-induced lethalities at the doses tested (data not shown). Virtually all lethal effects of the drug interaction studies were manifested within the first 30 min after PB administration. The time to reach death did not appear to be accelerated compared to that of PB administered alone.

Table 3. Percent lethality at 24 hrs for combinations of adrenergic drugs and PB alone or in the presence of atropine or atropine methyl nitrate.

Drug Interaction (mg/kg)	24 hr % Lethality	+ Atropine 24 hr % Lethality	+ Atropine Methyl Nitrate 24 hr % Lethality
epinephrine (5) + PB (3)	61.2	25.0	12.5
norepinephrine (7.3) + PB (3)	70.9	0	0
salbutamol (0.4) + PB (3)	62.5	0	0
yohimbine (1) + PB (3)	83.3	0	12.5
caffeine (5) + PB (3)	53.0	0	0

Mice pretreated with either atropine or atropine methyl nitrate exhibited a dramatically reduced incidence of lethality for epinephrine, norepinephrine, salbutamol, yohimbine or caffeine combined with PB, as shown in Table 3.

DISCUSSION

Pyridostigmine bromide is an anticholinesterase agent that has been used safely for decades to treat the neurological disorder known as myasthenia gravis. However, the recent implication of PB as a causative agent in Persian Gulf illnesses requires consideration of the potential modification of PB toxicity by concurrently ingested drugs or exposed chemicals, environmental variables and underlying pathological conditions. Previous studies have discussed the potential for adverse interactions between PB and anesthetic agents commonly used in surgery under

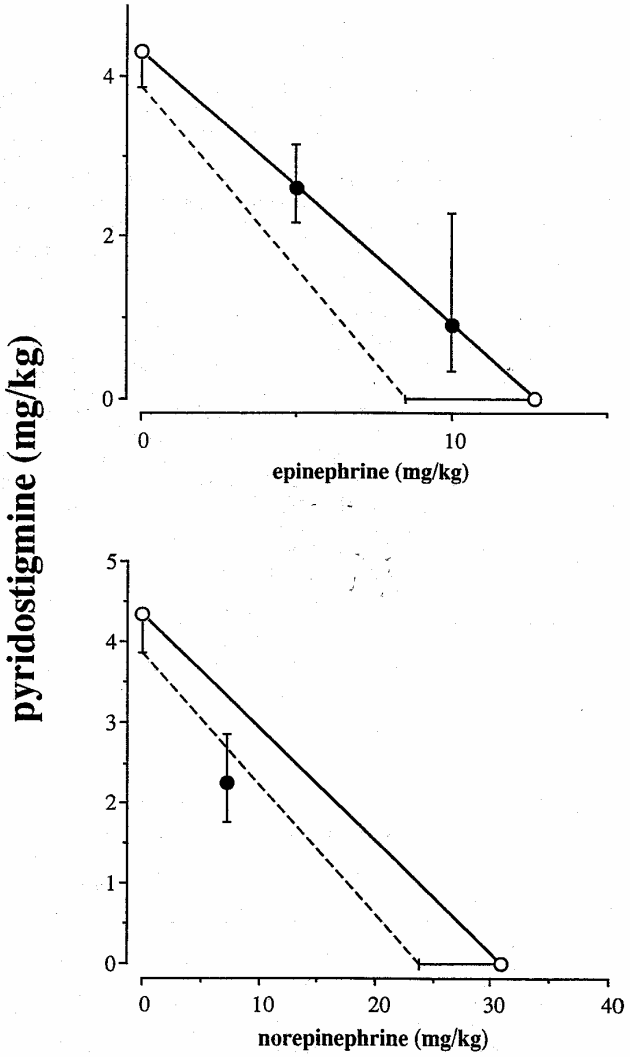

Figure 2. Isobolograms illustrating the combined lethal effects (LD_{50}) of PB with fixed doses of epinephrine (5 or 10 mg/kg) and norepinephrine (7.3 or 10 mg/kg) in mice. Symbols are the same as in Fig 1.

military conditions (21), but until now there have been no detailed reports of toxic interactions between PB and adrenergic drugs. The results of the present study demonstrate significant potentiation of acute lethality in mice when PB is combined with selected fixed doses of drugs that operate within the sympathetic nervous system to stimulate β-adrenoceptors, antagonize α-adrenoceptors or, presumably in the case of caffeine, cause release of catecholamines. Pretreatment with the muscarinic blocker atropine, or its corresponding quaternary ammonium derivative atropine methyl nitrate (22), diminished or abolished the lethal effect of these drug combinations. The ability of atropine methyl nitrate, which penetrates poorly into the CNS, to protect against these lethal effects suggests further that a peripherally mediated toxic interaction occurs following simultaneous exogenous activation of adrenergic and cholinergic receptor systems.

The mechanism through which the toxic potentiation operates remains unclear and is a subject for continued investigation. However, we hypothesize that the toxic potentiation observed between PB and drugs that alter adrenoceptor-related cell function relates to the production of an imbalance between peripheral adrenergic and muscarinic cholinergic control of cardio-respiratory events that leads to cardiac and/or respiratory arrest.

The administration of PB alone can be expected to enhance peripheral cholinergic receptor activation. This effect does not generally produce adverse effects (23) other than sinus bradycardia (24). Nevertheless, high oral doses of PB (100 mg/kg) have been reported to cause myocardial contraction band necrosis and to reduce activity of myocardial mitochondrial electron transport (cytochrome c oxidase) (25,26). It is of note also that intense vagal activation can cause myocardial structural damage (27) and that OP cholinesterase inhibitors in general (28), and PB in particular, have been documented to induce cardiac arrhythmias (29).

The more pertinent issue is whether combinations of drug agents or underlying pathologies can exacerbate the toxicity of PB. The present study examined drugs which increase function at adrenergic receptors, either directly or in the case of caffeine indirectly. Most of these agents are usually prescribed in the clinic at lower doses to produce cardiac stimulation, bronchodilation or control of blood pressure. Combinations of these agents with PB could result in an imbalance between peripheral adrenergic and cholinergic regulation of cardio-respiratory function, leading ultimately to cardiac and/or respiratory arrest. The cardiac impulse propagating/conducting system is a primary location for such detrimental interactions. Gotta and Sullivan (30) described excessive bradycardia or atrioventricular block in patients treated with PB during recovery from general anesthesia, a situation in which the sympatho-adrenal axis is stimulated. As discussed by Higgins et al (31), the interactions which occur between the parasympathetic and sympathetic nervous systems in the heart are complex, and responses to cholinergic interventions can be enhanced in the presence of increased sympathetic stimulation.

The administration of high doses of β-adrenoceptor agonists (isoproterenol, salbutamol, terbutaline) mimics defined aspects of activation of the sympatho-adrenal axis. In all cases sympatho-adrenal axis activation could be induced also as a result of drug-induced vasodilation. While no other data are available for isoproterenol or terbutaline and PB, precedent for interaction between salbutamol and PB in exacerbation of adverse respiratory responses does exist. Gouge and Daniels (32) conducted a study in which ingestion of a single 30 mg dose of PB by asthmatic soldiers during the Gulf War resulted in worsening of asthmatic symptoms. Eight of the 10 asthmatic participants in that study listed salbutamol (Albuterol MDI) as part of their normal therapeutic regimen. It should be noted that Caldwell et al (24) examined the effects of PB on the canine cardiopulmonary system and found that administration of PB alone resulted in marked increase in airway resistance.

The present results also demonstrate the potentiation of PB toxicity with co-administration of the non-selective α-adrenoceptor antagonist phentolamine, the α_1-adrenoceptor antagonist, prazosin and the α_2-adrenoceptor antagonist yohimbine. Phentolamine and prazosin can be expected to cause reflex increases in sympatho-adrenal outflow following drug-induced vasodilation, while antagonism of α_2-adrenoceptors by yohimbine blocks presynaptic autoinhibitory α_2-adrenoceptors, causing potentiation of norepinephrine release from adrenergic nerve terminals (14). It can be noted that Bagheri et al (33) have conducted studies with yohimbine in dogs which indicate that this agent may simultaneously activate both adrenergic and cholinergic nervous systems. Additional cholinergic activation by yohimbine could conceivably play a role in exacerbation of its toxic interaction with PB.

Caffeine is a psychomotor stimulant that is widely consumed at an average of 2.4 mg/kg/d by adults in the US (15). By blocking adenosine receptors, caffeine competitively inhibits the actions of adenosine to cause an increase in levels of circulating neurotransmitters, like acetylcholine, epinephrine and norepinephrine, which can also increase both adrenergic and cholinergic activity (15,34). At high doses, caffeine can indirectly elevate intracellular 3'-5'-cyclic adenosine monophosphate (cyclic AMP), an action also shared by β_2-adrenoceptor agonists (17).

It is unclear why epinephrine and norepinephrine administered at these doses did not potentiate lethality to the same extent as other agonist drugs, unless stimulation of α-adrenoceptors exerted a dominating protective effect. Buccafusco and Li (35) demonstrated that stimulation of α_2-adrenoceptors by clonidine limits the expression

of central toxicity following AChE inhibition by soman. Our studies support a protective role for clonidine since it failed to increase toxicity when administered prior to PB.

Although blockade of lethal effects by both atropine and atropine methyl nitrate would indicate a peripherally mediated mechanism of lethality in these studies, the possibility for CNS involvement in sublethal toxic effects could still exist. Friedman et al (9) reported indirect evidence that PB can enter the CNS under conditions of stress. In the brain, cholinergic and adrenergic neurons interact in the control of essential activities, such as cardio-respiratory function and cognitive performance.

Many of the reported symptoms of Persian Gulf illness, such as fatigue, ataxia and respiratory and gastrointestinal disturbances, resemble cholinergically mediated events (22). Poor compliance to PB dosing regimens during the Persian Gulf War could have resulted in some soldiers receiving larger acute doses of PB than are medically recommended. Information is not available as to the prevalence and usage of other medications by these individuals, or whether the extremely stressful wartime conditions played a role in their illness. While the relationship of the present findings to Persian Gulf illness remains to be established, it is clear that the concurrent usage of PB in individuals experiencing an enhanced sympathetic state should be investigated. Future studies should be directed toward elucidation of the actual mechanism of acute toxicity and the potential for non-lethal toxic effects to occur at lower, chronic doses of these drugs.

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REFERENCES

1. Breyer-Pfaff U, Schmezer A, Maier U et al: Neuromuscular function and plasma drug levels in pyridostigmine treatment of myasthenia gravis. J Neurol Neurosurg Psychiatry 53: 502-506, 1990.
2. Dunn MA, Sidell MD: Progress in medical defense against nerve agents. JAMA 262: 649-652, 1989.
3. Cook JE, Kolka MA: Chronic pyridostigmine bromide administration: Side effects among soldiers working in a desert environment. Mil Med 157: 250-254, 1992.
4. Persian Gulf Veterans Coordinating Board: Unexplained illnesses among Desert Storm veterans: A search for causes, treatment, and cooperation. Arch Intern Med 155: 262-268, 1995.
5. Haley RW, Kurt TL, Hom J: Is there a Gulf War syndrome? Searching for syndromes by factor analysis of symptoms. JAMA 277: 215-222, 1997.
6. NIH Technology Assessment Workshop Panel: The Persian Gulf experience and health. JAMA 272: 391-396, 1994.
7. Gavaghan H: NIH panel rejects Persian Gulf syndrome. Nature 369: 8, 1994.
8. Sutanto W, de Kloet ER: The use of various animal models in the study of stress and stress-related phenomena. Lab Anim 28: 293-306, 1994.
9. Friedman A, Kaufer D, Shemer J et al: Pyridostigmine brain penetration under stress enhances neuronal excitability and induces early immediate transcriptional response. Nat Med 2: 1382-1385, 1996.
10. McCain WC, Lee R, Johnson MS et al: Lethal interactive effects of acute oral exposure to pyridostigmine bromide, permethrin, and DEET in the laboratory rat. Toxicologist 30: 55, 1996.
11. Abou-Donia MB, Wilmarth KR, Jensen KF et al: Neurotoxicity resulting from coexposure to pyridostigmine bromide, DEET, and permethrin: Implications of Gulf War chemical exposures. J Toxicol Environ Health 48: 35-56, 1996.
12. Chaney L, Moss J, Mozingo J et al: Toxic interactions between pyridostigmine (PB), N,N-diethyl-m-toluamide (DEET), adrenergic agents and caffeine. Toxicologist 36: 21, 1997.
13. Moss JI: Synergism of toxicity of N,N-diethyl-m-toluamide to German cockroaches (Orthoptera: Blattellidae) by hydrolytic enzyme inhibitors. J Econ Entomol 89: 1151-1155, 1996.
14. Hoffman BB, Lefkowitz RJ: Catecholamines, sympathomimetic drugs, and adrenergic receptor antagonists. In Hardman JG, Limbird LE et al eds: Goodman and Gilman's The Pharmacological Basis of Therapeutics. McGraw-Hill, New York, NY: 199-248, 1996.
15. Benowitz NL: Clinical pharmacology of caffeine. Annu Rev Med 41: 277-288, 1990.
16. Libretto SE: A review of the toxicology of salbutamol (albuterol). Arch Toxicol 68: 213-216, 1994.
17. Borchard RE, Barnes CD, Eltherington LG: Drug Dosage in Laboratory Animals: A Handbook, 3rd ed. Telford Press, New Jersey: 1990.
18. Tallarida RJ, Murray RB: Manual of Pharmacologic Calculations with Computer Programs, 2nd ed. Springer-Verlag, New York: 153-159, 1987.
19. Gessner PK: Isobolographic analysis of interactions: an update on applications and utility. Toxicology 105: 161-179, 1995.
20. Noguchi Y, Sakai T, Nabata H et al: Prazosin hydrochloride. Oyo Yakuri 17: 39-56, 1979.
21. Keeler JR: Interactions between nerve agent pretreatment and drugs commonly used in combat anesthesia. Mil Med 155: 527-533, 1990.
22. Brown JH, Taylor P: Muscarinic receptor agonists and antagonists. In Hardman JG, Limbird LE et al eds: Goodman and Gilman's The Pharmacological Basis of Therapeutics. McGraw-Hill, New York: 141-160, 1996.
23. Arad M, Roth A, Zelinger J et al: Safety of pyridostigmine in hypertensive patients receiving beta blockers. Am J Cardiol 69: 518-522, 1992.
24. Caldwell RW, Lowensohn HS, Chryssanthis MA et al: Interactions of pyridostigmine with cardiopulmonary systems and their relationships to plasma cholinesterase activity. Fundam Appl Toxicol 12: 432-441, 1989.
25. Ito T, Akiyama N, Ogawa T et al: Changes in myocardial mitochondrial electron transport activity in rats administered with acetylcholinesterase inhibitor. Biochem Biophys Res Commun 164: 997-1002, 1989.
26. Kato T, Sugiyama S, Hanaki Y et al: Role of acetylcholine in pyridostigmine-induced myocardial injury: Possible involvement of parasympathetic nervous system in the genesis of cardiomyopathy. Arch Toxicol 63: 137-143, 1989.
27. Manning GW, Hall GE, Banting FG: Vagus stimulation and the production of myocardial damage. Can Med Assoc J 37: 314-318, 1937.
28. Kiss Z, Fazekas T: Arrhythmias in organophosphate poisonings. Acta Cardiol 34: 323-330, 1979.
29. Leenhardt A, Thomas O, Haouala H et al: Effet proarythmique de la pyridostigmine. Arch Mal Coeur 85: 1853-1856, 1992.
30. Gotta A, Sullivan C: A clinical evaluation of pyridostigmine bromide in the reversal of curarization. Can J Anaesth 17: 527-534, 1970.
31. Higgins CB, Vatner SF, Braunwald E: Parasympathetic control of the heart. Pharmacol Rev 25: 119-155, 1973.
32. Gouge SF, Daniels DJ: Exacerbation of asthma after pyridostigmine during Operation Desert Storm. Mil Med 159: 108-111, 1994.
33. Bagheri H, Chale JJ, Guyen LN et al: Evidence for activation of both adrenergic and cholinergic nervous pathways by yohimbine, an alpha$_2$-adrenoceptor antagonist. Fundam Clin Pharmacol 9: 248-254, 1995.
34. Carter AJ, O'Connor WT, Carter MJ et al: Caffeine enhances acetylcholine release in the hippocampus in vivo by a selective interaction with adenosine A1 receptors. J Pharmacol Exp Ther 273: 637-642, 1995.
35. Buccafusco JJ, Li W: Effectiveness of α_2-adrenergic receptor stimulation in reducing the central toxicity following cholinesterase inhibition. Res Commun Chem Pathol Pharmacol 75: 85-98, 1992.