Chapter Thirteen

NEUROTRANSMITTER DYSREGULATION

DOES PB ALTER REGULATION OF NEUROTRANSMITTERS, PARTICULARLY ACh?

Cholinergic Dysregulation

When ACh activity is artificially raised by administration of PB, it results in a host of compensatory changes, many of which act to reduce ACh activity. Changes in regulation of nerve signaling—occurring in response to excessive ACh activity—that result in reduced delivery of ACh or reduced response to ACh will be termed “downregulation.” (“Downregulation” is often used technically to refer to reduced production of receptors, but here it will be used to refer to any alteration in the system that produces low ACh activity—such as decreased production or release of ACh, reduced receptors or receptor-binding affinity or sensitivity, or increased breakdown of ACh.) More generally, regulation of ACh may be altered in ways that may not be exclusively downregulatory. For instance some elements of the system may experience abnormal heightened sensitivity to ACh. In the general case, there may be elements of the very complex ACh regulatory system that are downregulated, while other elements are upregulated, or simply abnormally regulated—and these alterations may themselves interact to produce system level changes in regulation. These changes will be referred to in the aggregate as “dysregulation.” In much of this discussion, downregulation—evidence of which is in the neuromuscular junction, as noted in the prior chapter—will illustrate how dysregulation could produce symptoms of altered ACh activity (low ACh activity in the case of downregulation), perhaps resulting in chronic symptoms if the changes outlast delivery of PB.

Based on the known functions of ACh—involving, for instance, muscle action, pain, sleep, and memory—such symptoms might include alterations in muscle function or muscular fatigue, altered sensitivity to or perception of pain, abnormal sleep, and disruption of learning and memory. These symptoms
appear to be compatible with symptoms prominently described by PGW veterans—the top symptoms reported by PGW veterans include fatigue, headache, muscle and joint pain, loss of memory, and sleep disturbances. Evidence favoring this hypothesis include known delivery of PB to many veterans, and demonstrated dysregulatory effects in muscle and brain in animal studies following delivery of PB and other AChE inhibitors. Most of the downregulatory effects for which a time-course has been evaluated are “mostly” reversible on discontinuation of PB (or other AChE inhibitors) in a relatively short time. Nonetheless, for many changes the time-course has not been characterized, and in some cases the normalization of function is not complete within the follow-up periods examined to date. Some changes have lasted as long following discontinuation of PB as anyone has looked.

Once again, although “downregulation” appears to occur for some elements of cholinergic function, the total picture may be more complex. For instance, reduced sensitivity of central nicotinic receptors following heightened cholinergic stimulation (a form of downregulation) may be accompanied by increased production of these receptors, perhaps as compensation to the compensation. (Although the increased production of receptors has been termed “upregulation,” the net effect of the several changes may still be depression of cholinergic function—or the net effect could be “supersensitivity.”) Further complicating the picture is the possibility that changes in the ACh system will engender additional changes in neurotransmitter systems regulated by, or interacting with, the ACh system.

One might suppose that the relative contributions of up- and downregulation would be easy to tease apart. This is not necessarily the case. In myasthenia gravis, symptoms of the disease—involving low ACh action at the muscles—are often difficult to distinguish from symptoms of overtreatment—involving high ACh action at the muscles. Both produce weakness. Although regulation shifted “up” and “down” may be perceived as “abnormal,” the valence of the abnormality may not always be easy to discern. (Challenge tests—involving administration of pro- or anticholinergic agents—would be expected to worsen or ameliorate symptoms, depending on whether excessive or reduced ACh activity took place. Such challenge tests may be helpful if simple up- or downregulation has occurred or has predominated.)

Many limitations remain in the “downregulation” (or “dysregulation”) hypothesis. For instance: Although changes consistent with downregulation (dysregulation) have been demonstrated in animal studies, most of these changes have been shown at the neuromuscular junction, which is the most studied site. PB normally is denied access to the brain, and unless PB is admitted centrally, effects on learning, memory, attention, and sleep might not be expected. Most studies have used higher doses of AChE inhibitors, leading to more substantial
inhibition of AChE than that experienced by PGW veterans, and it is not known whether the relatively low dose of PB—with the accordingly lower amount reaching the brain—is compatible with downregulation (dysregulation) effects. Finally, it is not known whether such downregulation (dysregulation) effects, assuming they could be produced by such doses, could last sufficiently long (or evolve in such a manner) to account for such chronic symptoms as those described by ill PGW veterans.

**WHAT IS DOWNREGULATION?**

Many different mechanisms regulate signaling by chemicals in the body. When drugs or chemicals are given that abnormally elevate (or depress) delivery of and response to signals by body chemicals, compensatory changes occur in tissues in the body to counteract the abnormally high (or low) signaling produced by these drugs. When drugs abnormally increase signaling, the compensatory effects that occur and that produce attenuation of this heightened signaling may be termed “downregulation.” (At the signal-receiving side, effects—such as reduced sensitivity of receptors to ACh—may be termed “subsensitivity.”) The corresponding clinical or behavioral effects reflecting the reduced response by the body to the drug are termed “tolerance.” The clinical effects of reduced response by the body to the native signaling chemical after discontinuation of the drug may be termed “rebound.”

“Tolerance” is familiar to physicians, including tolerance to the effects of nitrates, sleep medications, or opiates. Exposure of catecholamine-sensitive tissues to adrenergic agonists results in progressive attenuation of their ability to respond; mechanisms for these phenomena (termed “desensitization,” “refractoriness,” or “tachyphylaxis”) are incompletely understood but multiple points of regulation appear to include receptors, G proteins, enzymes including adenylate cyclase, and cyclic nucleotide phosphodiesterase, with the pattern of refractoriness varying according to the extent that the different components are modified (Hardman, Limbird, et al., 1996). Acute “rebound” phenomena on discontinuation of a drug, which act in the reverse direction of the drug (due to development of downregulation in the signaling system(s)) are also widely known. These include rebound hyperadrenergic effects following discontinuation of beta blockers or rebound insomnia following discontinuation of chronically or subacutely administered sleeping pills. (The consequences of cessation of nicotine administration in persons who stop smoking provides clear evidence of long-term consequences of cholinergic modification.)

For some substances, dramatic rebound effects occur early following withdrawal—such as the hypersomnolence following withdrawal of cocaine, the dramatic withdrawal syndrome following cessation of opiates, or even the
withdrawal syndromes seen with cessation of selective serotonin reuptake inhibitors (Dominguez and Goodnick, 1995; Rosenstock, Keifer, et al., 1996; Haddad, 1997; Lejoyeux and Ades, 1997). These effects may contribute to the addiction process for some drugs. However, over the long term, somewhat more subtle but clinically important “rebound” effects may also ensue. Instances include the long-term effects following cessation of corticosteroids and opiates. For these drugs, withdrawal leads to far more long-standing, comparatively subtle changes that may be evident only in selected contexts. Physicians who care for former narcotics addicts are aware that such patients experience heightened sensitivity to pain (where pain constitutes the “challenge” to the opiate system), which may persist many years following cessation of opiates, perhaps even for life. Analogously, an inadequate corticosteroid response to major stress (such as the stress of surgery) is present for a highly variable duration (Wilson, Braunwald, et al., 1991)—from days up to a year, after as little as a week of pharmacological corticosteroid treatment; for this reason, physicians must remember to administer “stress doses” of corticosteroids if a major stressor is encountered during this time. Thus, different downregulatory effects occur, with different time-courses ranging from days to years. Indeed, several different time-courses may characterize different effects for the same drug.1

DOWNREGULATION IN THE ACh SYSTEM

Downregulation is also known to occur in the acetylcholinergic system and indeed has been cited as a factor that must be considered with respect to drug interactions in military personnel who have taken PB and who may require drugs as part of surgery for traumatic injury (Keeler, 1990). Regarding effects at the tissue level, there exists evidence from animal studies that changes in tissues result from excessive activity of ACh following administration of PB. In many cases these changes are “downregulatory,” serving to counteract the excess acetylcholinergic activity while it occurs, leading to development of “tolerance” both to the therapeutic benefits (in myasthenia) and to the toxic effects, despite continued AChE inhibition. Downregulatory effects may include presynaptic effects, such as reduced production of ACh (fewer packets of ACh or less ACh in each packet); reduced release of ACh; or withdrawal of

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1Nonpharmacological manipulations that produce a major surge or other alteration of signaling chemicals may also have lingering repercussions. Thus, rapid eye movement (REM) deprivation leads to REM rebound (the time-course has not been well characterized, but is believed to be on the order of several weeks). Neuroendocrine dysfunction produced by undernutrition or anorexia nervosa may persist despite complete recovery of body weight loss (Falk and Halmi, 1982). And serious psychological stressors, presumably at least in part through their neurochemical effects, may lead to lasting changes in neurochemical regulation in the condition termed Posttraumatic Stress Disorder (PTSD) (see section on “Stress”).
cholinergic nerve terminals from their contacts. Postsynaptic effects may include reduced number or “density” of ACh receptors, reduced affinity of receptors for binding to ACh, or reduced sensitivity of receptors to the actions of ACh once bound. Moreover, increased breakdown of ACh (such as by production of increased AChE) may occur. These mechanisms may have different distributions—they may differ according to specific brain regions (or muscles); according to different “layers” of neurons, e.g., within the cortex, within a brain region; and according to the type of ACh receptor, contact cell, or AChE molecule. (In fact, effects may be in opposite directions for different brain regions, receptor types, etc.) Moreover, the varying changes may have different time-courses of appearance and of resolution. They may even lead to alterations in susceptibility to future ACh system exposures.

**Data from the Neuromuscular Junction: Peripheral Nicotinic Receptors**

Most of the basic-science evidence regarding downregulation derives from investigation of the neuromuscular junction and is reviewed in the chapter devoted to that issue (Chapter Twelve). (Additional discussion of certain elements of “downregulation” appears in the Chapter Fourteen, “Chronic Effects.”) Changes that appear to take place at the neuromuscular junction following administrations of PB and other AChE inhibitors include presynaptic effects—such as withdrawal of nerve terminals, reduction in number of ACh quanta released following a nerve impulse, and reduction in ACh per quanta—and postsynaptic effects, such as reduction in receptor density and reduced receptor sensitivity. Just as excessive ACh action leads to downregulation, so abnormally depressed ACh action leads to upregulation; for instance, experimental denervation has been shown to increase receptor synthesis while stimulation suppresses it (Heinemann, Asouline, et al., 1987). Such changes serve to attenuate the effects of excessive ACh resulting from administration of PB or other AChE inhibitors while these agents continue to be given. Such changes would be expected to produce low acetylcholinergic activity once PB is withdrawn. Although the time-course of this altered regulation has not been characterized—that is, the speed with which it returns to normal following cessation of PB is not known—in studies in rats, at least some changes observed in the muscle or the neuromuscular junction have persisted even 60 days after termination of PB treatment, which is the farthest out such studies have been conducted (see Chapter Twelve, “Neuromuscular Junction”). Different effects appear to have different time-courses, even with the same drug—be it PB (see Chapter Twelve) or soman (Russell, Booth, et al., 1986). Functional manifestations of downregulatory effects, in the form of tolerance, are variable. However, these include a (mostly reversible) dose- and frequency-dependent

As has been stated, most evidence regarding downregulation and tissue “subsensitivity” derives from the neuromuscular junction. This is in large part a consequence of the neuromuscular junction being the best studied and best understood of all synapses, “because of its accessibility to biochemical and electrophysiological techniques” (Heinemann, Asouline, et al., 1987); likewise, the muscle nicotinic ACh receptor is “the best characterized neuroreceptor to date” (Goldman, Evans, et al., 1987). Evidence is substantially less rich regarding nicotinic receptors present at other sites, or muscarinic receptors.

Data from Central (Brain) Muscarinic Receptors

Although downregulation is best documented at the neuromuscular junction, there exists some evidence to indicate that some such downregulatory changes may occur in the brain, and in the muscarinic system in particular (data from rodents) (Costa, Schwab, et al., 1982a; Schwab, Costa, et al., 1983; Stanton, Mundy, et al., 1994; Russell, Booth, et al., 1989), following administration of AChE inhibitors, including organophosphorus agents and carbamates (Costa, Schwab, et al., 1982a and 1982b). Administration of AChE inhibitors—e.g., soman—has been shown to lead to strongly reduced synthesis rate of ACh (Nordgren, Karlen, et al., 1992). Additional mechanisms of tolerance in the muscarinic system include receptor loss (Uchida, Takeyasu, 1979; Schiller, 1979; Schwab, Hand, et al., 1981; Schwab and Murphy, 1981; Ehler, Kokka, et al., 1980a, 1980b; Costa, Schwab, et al., 1981; McPhillips, 1969; Brodeur and Duboi, 1964; Russell, Overstreet, et al., 1975; Schwab, Costa, et al., 1983); however, tolerance in some experiments has been detected prior to demonstration of reduced receptor number, indicating the presence of other mechanisms (Marks, Artman, et al., 1981; Schwab, Costa, et al., 1983). One apparent change is enhanced affinity of AChE for ACh (so that ACh is more likely to be broken down), which has been seen both peripherally and centrally following administration of an irreversible AChE inhibitor (Milatovic and Dettbarn, 1996). Some additional mechanisms have been proposed (El-Fakahany and Richelson, 1980, 1981), and many other remain unstudied (Schwab, Costa, et al., 1983). The relevance of these data to PGW veterans is limited by use of animal subjects (primarily rodents) and by the fact that the AChE inhibitors used were not PB. The effects differ from one tissue to another—and indeed, may be prominent in one tissue (e.g., ileum) and absent in another (e.g., heart) (Schwab, Costa, et al., 1983), a reminder that examination of one type of tissue may not be sufficient to ensure or exclude effects of this kind in other tissues. Regarding the issue of whether these changes might be prolonged, a single subcutaneous dose of
chlorpyrifos led to long-lasting enhanced sensitivity to cholinergic antagonists (evidenced by exaggerated hyperactivity in response to scopolamine) in rats, which persisted for months although muscarinic receptor density and cholinesterase activity had returned to normal levels (Pope, Chakraborti, et al., 1992). Similarly, a single dose of fenthion led to apparently permanent alterations in intracellular communication of muscarinic receptors studied in rats (Tandon, Padilla, et al., 1994; Tandon, Willig, et al., 1994).

**Data from Central (Brain) Nicotinic Receptors**

Data regarding downregulation and central nicotinic function are complex. Nicotinic receptors are present not only in the neuromuscular junction but also in the CNS, as well as on autonomic ganglia and chromaffin cells in the adrenal medulla, where they participate in regulation of catecholamine release (Heinemann, Asouline, et al., 1987; Hardman, Limbird, et al., 1996). However, the brain receptors are not identical to those at the neuromuscular junction; for example, the receptors are not blocked by exactly the same complement of chemicals (Luetje, Patrick, et al., 1990). Indeed, many types of nicotinic receptors are in the CNS; the exact number is unknown, but it has been speculated that the CNS “might contain a large set of nicotinic receptors, each having different properties” (Heinemann, Asouline, et al., 1987). Certainly a number of pharmacologically different nicotinic receptors have been identified, with different distributions in the brain (Wada, Balivet, et al., 1987; Wada, Wada, et al., 1989; Wada, McKinnon, et al., 1990; Patrick, Sequela, et al., 1993; Luetje, Patrick, et al., 1990). Not only are there different types of nicotinic receptors, but, further complicating the ability to extrapolate evidence with confidence from the neuromuscular junction, more than one type of nicotinic receptor can exist in one region (Alkondon and Albuquerque, 1993) or even on one cell type (Heinemann, Asouline, et al., 1987). There is some literature on the effects of stimulation of central nicotinic ACh receptors, much of which derives from efforts to understand nicotine addiction; but it appears that nicotine itself is a special case. It has been said that “there are many examples of receptor changes in the brain following repeated administration of drugs that increase or decrease receptor stimulation either directly or indirectly. These changes in

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2The receptors are proteins composed of five subunits (Patrick, Sequela, et al., 1993). There are 11 known members of the gene family that encodes the subunits of the neuronal nicotinic ACh receptors: the genes and their products include alpha2–alpha9, beta2–beta4, so that many combinations are possible (Elgoyen, 1994; Patrick, Sequela, et al., 1993). Though not all possible combinations have been described, at least nine different receptors resulting from combinations of alphas with betas—or alpha alone—have been demonstrated (Duvoisin, Deneris, et al., 1989; Patrick, Sequela, et al., 1993). Those nicotinic receptors that occur at the neuromuscular junction are more homogeneous, containing two alphas, a beta, a gamma, and a delta; or the gamma may, depending on animal species and cell maturity, be substituted by an epsilon (Alkondon and Albuquerque, 1993).
receptors are usually reciprocal to the changes in stimulation, resulting in a pattern consistent with compensatory adaptation to the change in stimulation” (Schwartz and Kellar, 1983). This pattern (consistent with downregulation) is slightly modified in the case of nicotine, as we shall see, but the essential effect is apparently the same.

**Factors in Downregulation.** When agents that activate nicotinic receptors—other than nicotine—repeatedly stimulate nicotinic receptors, it leads to a reduction in receptor number—that is, receptor “downregulation.” Administration of drugs—like AChE inhibitors—that cause “excessive” activation of muscarinic receptors leads to reduced numbers of muscarinic receptors, apparently as compensation, as noted above. A similar phenomenon is seen with activation of nicotinic receptors—except with nicotine itself. DFP (diisopropyl fluorophosphate) is an AChE inhibitor (as is PB), which—like other AChE inhibitors—produces a net increase in ACh action. In live mice, ten days of exposure to this agent (1 mg/kg tapered to 0.2 mg/kg daily under the skin) produces reduced nicotinic binding sites—that is, reduced density of nicotine receptors—consistent with the idea that receptor downregulation occurs after heightened nicotinic receptor activation (Schwartz and Kellar, 1983; Schwartz and Kellar, 1985). (For nicotine, it appears that depression of the nicotine system as a whole may occur following stimulation by nicotine, but receptor number may increase.) Thus, the effect on nicotinic receptors with DFP is more like the effect seen with muscarinic receptors—that is, reduced receptor number following activation.

Testing of carbamylcholine (also called “carbachol”), an “agonist” or stimulant of the ACh system, has been done in tumor cells from a clone of a type of tumor called a “pheochromocytoma”; these cells possess the type of nicotinic ACh receptors that are found on neurons (Robinson and McGee, 1985; Simasko, Soares, et al., 1986), and they serve as a “model” of neuronal-type nicotinic ACh receptors. (This is in contrast to the above studies, done in actual animals, looking at effects on their nicotinic receptors in vivo or in vitro). In addition to classically described desensitization of the receptors, a second process was found to occur that produced a nonrecoverable loss of neuronal ACh receptor activity. This process was termed inactivation (Simasko, Soares, et al., 1986). The onset was slower than for desensitization (15 minutes rather than three-fourths of a minute), depended on the concentration of carbachol, and was blocked by giving nicotinic antagonists (which prevented the carbachol from binding and having action on the nicotinic receptors). (Of note: Desensitization and receptor downregulation occur at a higher dose of carbachol than the one that produces maximum stimulation. The result is a biphasic response, with the response to carbachol increasing to a certain point, and then decreasing (Robinson and McGee, 1985). Thus, in some instances a monotonic “dose
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response” effect may not be seen.) Recovery was not seen in recovery buffer (over a two hour period). Inactivation did not seem to require desensitization (since strategies to reduce desensitization did not affect inactivation). It was suggested that inactivation might be a first step to receptor downregulation and could explain the rapid and prolonged tolerance to effects of nicotinic “agonists” (agents that bind and stimulate the nicotinic receptors) (Simasko, Soares, et al., 1986).

Factors in Upregulation. When nicotine binds to the nicotinic type of ACh receptor, there is an increase in the number of nicotinic ACh receptors—receptor upregulation. Nonetheless, the nicotinic ACh system as a whole appears to be depressed. Nicotine is an agent that binds readily to the “nicotinic” ACh receptors, activating them. Repeated or chronic exposure to nicotine, rather than reducing the number of ACh receptors, increases the number (Marks, Pauly, et al., 1992) (although there is no change in the affinity of receptors for nicotine—that is, in their inclination to bind to nicotine) (Marks and Collins, 1983). At least this is true for some types of receptors (Flores, Rogers, et al., 1992), in some parts of the brain (Pauly, Marks, et al., 1991). This seems to be in contrast to what occurs with activation of nicotinic receptors by other agents, and with activation of muscarinic receptors, in which receptor downregulation occurs. The special effect of nicotine may result from the ability of nicotine to desensitize or inactivate nicotinic receptors; the result is not fundamentally inconsistent with nicotinic system “downregulation” (in our use of the term). Once again, the effects that occur vary widely by area in the brain. Finally, the consequences have widely varying time-courses. Since effects differ by area of the brain and in time-course, there may be altered interactions among brain areas in consequence.

Following nicotine administration in animals—studies have mostly been done in mice and rats—the number of nicotinic receptors increases. Yet the nicotinic activity as a whole seems to be dampened rather than heightened following nicotine administration (nicotinic system “downregulation” in our terminology)—despite the increase in the number of nicotinic receptors. The most important evidence derives from the effects of later administration of nicotine. Treatment with nicotine for a time has been shown to abolish or reduce the response to nicotine given later, for a variety of responses, producing (in rodents) tolerance to effects of nicotine such as release of prolactin and of adrenocorticotropic hormone, depression of locomotion or “Y-maze activity” and rears, reduction in body temperature, change in heart rate, and effects on “rotarod” performance (Marks and Collins, 1983; Marks, Stitzel, et al., 1985; Collins and Marks, 1988; Hulihan-Giblin, Lumpkin, et al., 1990; Pauly, Marks, et al., 1991). (It has been observed that the “acute and chronic administration of nicotine might induce changes in central nicotinic cholinergic circuits that
How could there be an increase in receptors, and yet reduced response? This could occur if the increase in receptors were in turn partial compensation for reduced response occurring by some other mechanism. Nicotine appears to cause desensitization (or inactivation (Hulihan-Giblin, Lumpkin, et al., 1990; Pauly, Marks, et al., 1991)) of nicotinic ACh receptors. Indeed, one study—published in abstract form—has shown that prolonged incubation with low levels of nicotine, while causing little receptor activation, effectively blocks the nicotinic acetylcholinergic receptors, by stabilizing the desensitized state(s) of the receptor (Lester and Dani, 1994). This abstract alludes to the nicotinic receptor upregulation that also results—perhaps as a consequence of the desensitization. Tolerance may arise from such desensitization—and increased receptor number could result as a compensatory response to that effect. Indeed, chronic nicotine treatment results in an increase in nicotinic receptor number that parallels (and may be caused by) a decrease in sensitivity to nicotine (Schwartz and Kellar, 1985; Collins and Marks, 1988).

Tolerance (net “downregulation” of the system—though not of the receptors) results if the increase in receptor number is exceeded by the increase in number of receptors that are inactivated (Collins and Marks, 1988)—otherwise stated, if the increase in receptor binding by nicotine is more than offset by a loss in effect of binding. In fact, the number of binding sites may be inversely (rather than positively) related to effects produced by nicotine—the more receptors, the less the effect (Collins and Marks, 1988)—consistent with production of tolerance. Chronic treatment with nicotine leads to an increase in density of nicotine binding sites, in some but not all areas of rat brain (for instance, in the frontal cortex but not the striatum): If it is true that the increase in number of receptors accompanies—and may perhaps be secondary to—a reduction in measured aspects of function, then the same brain areas that show reduced function (such as nicotine evoked release of dopamine) should be those with increased receptor density, and indeed this appears to be true (e.g., again, frontal but not striatal), consistent with the possibility that the rise in receptor number is secondary to—and lesser in effect than—the reduction in receptor sensitivity (Wonnacott, Marshal, et al., 1994).

It has been suggested that strains (or individuals) that show more tolerance to nicotine may have receptors that resensitize slowly, whereas strains that develop tolerance less readily may have receptors that desensitize poorly or resensitize rapidly (Collins and Marks, 1988). (Actually, the story is undoubtedly more complex than this. For instance, increases in the number of receptors were seen before development of detectable tolerance to a challenge with nicotine; the attenuation by nicotine of stress-induced prolactin release...
appears to be independent of nicotine-induced desensitization to the stimulatory effect of subsequent injection of nicotine on prolactin secretion; and correlations between binding and acquisition and loss of tolerance to effects of nicotine vary according to which effect is being examined, indicating that other explanations for tolerance must be sought as well (Marks, Stitzel, et al., 1985; Sharp, Beyer, et al., 1987.)

In another way of characterizing similar information, some theorize that the time-averaged effect of nicotine is as an antagonist rather than an agonist (Hulihan-Giblin, Lumpkin, et al., 1990)—that is, it may do more, over time, to block activation of nicotinic receptors than to activate them. For instance, after a single injection of nicotine into rats, the prolactin response to a later injection is smaller; the investigators took this to mean that “nicotine is even more potent in stimulating desensitization of nicotinic cholinergic receptors than in stimulating prolactin release”; that after brief activation it causes protracted desensitization or inactivation of the receptors so that its “predominant” effect is that of an antagonist (Hulihan-Giblin, Lumpkin, et al., 1990). It has also been speculated that antagonist effects could occur perhaps through metabolites of nicotine, such as cotinine. Cotinine competes for ACh binding sites in the brain; and one or more metabolites of nicotine (possibly hydroxynicotine) confer protection against the lethal effects of nicotine in mice, which may support this idea (Schwartz and Kellar, 1983). Either way, it appears that increase in receptor number may be the consequence of reduced action of the nicotinic system, which in turn follows (and perhaps follows from) the increased activity initially provoked by nicotine.

Could some receptor types show a net increase in action resulting from nicotine? It remains possible, though the present literature review failed to uncover substantial support for this. Certainly, in instances in which ACh stimulation drives something else that is inhibitory, suppression of ACh could lead to stimulation of that other action.

Regional Brain Differences and Differences Between Types of Nicotinic Receptors. As mentioned, it has been shown that there are large differences in the effect (the increase in receptors—and perhaps the reduced sensitivity of receptors—in response to nicotine) in different parts of the brain. A study in mice found that in most brain areas, chronic nicotine infusion increased the number of nicotinic receptors (sites to which nicotine attached), but the extent of the increase and the dose response curve (how big of a dose is needed to provide how big of an increase) were “widely different among brain regions” (Pauly, Marks, et al., 1991) with some regions particularly “resistant” to change. Also, the type of nicotinic receptor may determine the degree of receptor change, and of functional tolerance. Binding by the agent alpha bungarotoxin (which binds to some nicotinic receptors but not to other types) was significantly
increased in only 26 of 80 regions examined (Pauly, Marks et al. 1991), even at the highest dose, so that different receptor types behave differently, as well. Indeed, the point has been carefully made that these effects may well differ from one type of nicotinic receptor to another (Lester and Dani, 1994). Moreover, brain regions are not equivalent in nicotinic cholinergic regulation after stimulation with nicotine—for reasons due to and/or distinct from the types of nicotinic receptors in residence.

**Individual Differences in Nicotinic Regulation.** In animals, there are large differences from one strain to another in sensitivity to nicotine—and moreover in ability to develop tolerance to nicotine (Collins and Marks, 1988)—reinforcing the idea that there may be within-species individual differences in regulation of the ACh system. Strains that are high or low in nicotine binding (to one type of receptor) in certain brain regions are likely to be high or low in other regions—though binding to one type of receptors correlates poorly with binding to the other type (Collins and Marks, 1988).

The time-course of recovery from tolerance may be highly variable. Tolerance to the effects of nicotine has been shown to be lost at different rates for different tests in rodents. For instance, loss of tolerance (return to baseline or near baseline) occurred for Y-maze activity and rearings test in eight days; regaining control of body temperature required 12–16 days; and tolerance for a heart rate test persisted throughout a 20 day withdrawal period—although brain binding sites had returned to control levels by eight days after treatment, and alpha-bungarotoxin binding sites (representing a subset of nicotinic receptors) were normal at the first time tested (four days). This indicates that physiologic effects may persist after objective identified measures have returned to normal.3

Findings in humans appear consistent with findings in animals. It is not easy to study the brains of humans, at the cellular and subcellular level, while they are still alive, but studies have been done postmortem to compare nicotinic receptors (binding sites) in people who smoked with those in people who did not smoke. Consistent with findings in animal studies, smokers—presumably exposed chronically to nicotine—showed an increase in nicotine binding in an assortment of brain areas (like the hippocampus, cerebellum, and median raphe nuclei—involved in serotonin production)—but not in all areas (for

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3Presumably these measured physiologic effects are consequences of neurobiological factors that should have objective measurable underpinnings, though it is not yet known what these objective correlates are and how to measure them. The fact that objective measures that correlate with the demonstrated physiologic effects have not been identified does not imply that mechanisms do not exist. This important point bears reflection when assessing symptoms in ill PGW veterans. A search for objective measures sensitive to the complaints of veterans must remain a priority, because absence of effect on existing tests can never be assumed to imply absence of organic mechanism.
instance the medulla oblongata) (Benwell, Balfour, et al., 1988). As in animals, the increase in binding appeared to result from an increase in number of receptors rather than an increase in affinity for receptors (Benwell, Balfour, et al., 1988). These findings corroborate that at least some of the findings from animals can be extended to people, including the variability of the effects from one part of the brain to another.

The Case of ACh Receptors in General. Clinically, there is indeed induction of “tolerance” with AChE inhibitors (Blick, Weathersby, et al., 1994; Russell, Overstreet, et al., 1975; Rider, Ellenwood, et al., 1952; Sterri, Lyngas, et al., 1980; Schwab and Murphy, 1981; Bombinski and DuBois, 1958; Barnes and Denz, 1954; Smit, Ehlert, et al., 1980a, 1980b), including reduced clinical response to the same dose, such as reduced evidence of AChE inhibitor toxicity with later doses and with consistent inhibition of AChE, reduced response to subsequent higher doses following pretreatment with low doses, and reduced response to a later similar chemical (Schwab, Costa, et al., 1983). For example, the dose of the (AChE-inhibiting) nerve agent soman required to produce a performance decrement on a specific well-learned task in rhesus monkeys was two and a half times higher with continued use (e.g., more than five days) than initially; and required a substantially greater degree of AChE inhibition, reflecting development of tolerance to low levels of AChE activity (Blick, Weathersby, et al., 1994). Indeed, “tolerance” to the effects of PB is reported for patients with myasthenia gravis, who may require progressively increased doses (McEvoy, 1991); and is suggested by reports in PGW veterans, in some of whom side effects attributed to PB abated with continued use (Sharabi, Danson, et al., 1991).

Evidence of the effects of AChE inhibitor administration on later response to ACh agonists suggests reduced sensitivity. Evidence on the effects of AChE inhibitor administration on later response to ACh antagonists is less consistent. Some data relating to use of carbamates suggest that enhanced sensitivity to antagonists accompanies reduced sensitivity to agonists (Costa, Schwab, et al., 1982a and 1982b), amplifying the “downregulation” effect. Another study found no enhanced sensitivity to atropine (a muscarinic antagonist) after administration of PB and suggested this may reflect the relatively low doses of PB used in military prophylaxis (Matthew, Glenn, et al., 1993). Other evidence suggests that binding of antagonists, as well as agonists, may be reduced following AChE inhibitor, and affinity for binding may remain unchanged (Schwab, Costa, et al., 1983). Thus, the net effect of AChE inhibitor administration on response to ACh antagonists may differ according to which of a set of effects predominates, including reduced receptor number, unchanged or enhanced receptor affinity, and perhaps other consequences of AChE inhibitor administration. Supersensitivity, rather than downregulation,
may occur with some receptor types in some conditions (Nilsson-Haransson, Lai, et al., 1990).

At the time this report was first circulated (including this chapter on dysregulation), ACh system dysregulation had not been previously proposed as a mechanism to consider in relation to illness in PGW veterans. Since then, additional evidence is emerging suggesting that such dysregulation may occur. More recent findings are included in an Addendum.

**PREDICTIONS FROM THIS HYPOTHESIS**

This hypothesis suggests that symptoms in ill PGW veterans should bear some relation to effects mediated by the ACh system.

**General Comments.** Both tissue and behavioral studies support the development of downregulation—or more generally dysregulation—of the acetylcholinergic system following administration of AChE inhibitors. Although existing evidence suggests that different ACh receptors, and therefore different functions of the ACh system, may alter their regulation in different ways and with different time-courses, existing evidence is insufficient to predict what functions should be influenced how. Overall, however, if symptoms result from cholinergic dysregulation, many or most should relate to functions under control of the ACh system. It remains to be asked, what might the expected consequences of such dysregulation be? Regulation of ACh turnover and concentration in synaptic junctions and neurons is believed to play an important role in conditions involving memory dysfunction (e.g., Alzheimer’s disease), motor dyscontrol (e.g., tardive kinesia), and muscular fatigue (e.g., myasthenia gravis) (Lieske, Gepp, et al., 1993). The consequences of ACh dysregulation are at once potentially varied and dependent on the specifics of the dysregulation, with expected symptoms determined by the site and character of the cholinergic alteration.

Effects might be expected to occur in the periphery, due to presumably limited access of PB to the brain. However, central consequences could ensue if PB

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4If PB does not gain access to the brain, it is not inconceivable that some central effects could occur indirectly, through modulation of the peripheral nervous system, which interacts with and influences the CNS. Nicotinic receptors occur in the adrenal medulla and are involved in regulation of catecholamine release from chromaffin cells (although, in fact, catecholamine release by physostigmine appears to be a predominantly central process—as discussed elsewhere). Just as cholinergic function is subject to downregulation, so is the catecholamine system: exposure to catecholamines leads to reduced responsiveness of the catecholamine system, involving multiple points of regulation (Hardman, Limbird, et al., 1996). Enhanced exposure to catecholamines might occur through administration of PB, because PB would presumably increase ACh binding to nicotinic receptors in the adrenal medulla, producing increased catecholamine response and increased susceptibility to catecholamine downregulation. This effect would be heightened in circumstances of...
Neurotransmitter Dysregulation does gain central access. This possibility is suggested by the high prevalence of apparently central symptoms that occurred in response to PB reported by Gulf War personnel (Sharabi, Danon, et al., 1991), as well as by some evidence from animals suggesting enhanced access of PB to the CNS in conditions of stress (Friedman, Kaufer, et al., 1996). In fact, because no troops in the same units were assigned to not receive PB, with whom PB recipients could be compared, it cannot be stated with certainty that the CNS symptoms were in response to PB. However, personnel interpreted these effects as occurring in response to PB, and effects were consistent with central effects of AChE inhibition, discussed in Chapter Three, and with side effects of PB reported by patients with myasthenia gravis, in whom some central effects of PB are reported (Hood, 1990).

Likely Impaired Functions. Functions known to be governed by the cholinergic system, in general, should be those most likely impaired by cholinergic downregulation or dysregulation. These include sleep, learning and memory, nociception (pain perception, in which ACh serves in a modulatory role) (Sitaram and Gillin, 1980), and neuromuscular action. Indeed, prominent among symptoms reported by PGW veterans are problems with memory, sleep, pain, and musculoskeletal dysfunction. These are general terms, involving functions controlled by multiple mechanisms. A certain degree of greater specificity is desired; in the case of sleep, REM sleep in particular is governed by ACh and should be an object of scrutiny. (As noted later, sleep apnea may also have a cholinergic relation.) Where possible, more specific characteristics of the general functions that are under cholinergic control should be identified for investigation in PGW veterans.

Specific functions affected by cholinergic agents in normal people have been reported to include increased REM sleep (Sitaram and Gillin, 1980), and reduction of the P100 component of the visual EEG evoked response was reported. The “opposite” phenomena would be expected if downregulation has occurred and has produced effects uniformly opposite those of the specific cholinergic agonist used in those studies. These effects would be expected to include reduced REM, reduced arousal (perhaps having a clinical correlation in chronic fatigue or perhaps not), enhanced perception of pain, and reduced serial learning and memory (retrieval of clustered information).

stress, because the stress response itself would lead to enhanced release of catecholamines. Most symptoms reported by PGW veterans with PB administration appear more consistent with ACh system effects rather than peripheral catecholaminergic enhancement. Although it is conceivable that some delayed abnormalities in ill PGW veterans could involve dysregulation of the catecholamine system, neither the time-course of effects nor the nature of most reported effects seems closely consistent with this representing a major factor. (However, direct studies of autonomic function would be needed to preclude this possibility.)
Effects on mood would also be expected, though the direction of these effects is difficult to anticipate. The AChE inhibitor DFP may produce depression or lead to remission of mania, and its withdrawal may lead to precipitation of mania (Janowsky, El-Yousef, et al., 1974). Moreover, other evidence has suggested a “cholinergic supersensitivity” theory of affective disorder, suggesting that muscarinic upregulation and supersensitivity are associated with depression (O'Keane, O'Flynn, et al., 1992). (However, this may be a case of low baseline cholinergic action with concomitant heightened receptor expression.)

This might predict reduced incidence of depression in PGW veterans compared to controls with comparable health and environmental circumstances. If positive mood effects occur, they might be counteracted by factors that depress mood, such as ill health and enhanced sensitivity to pain. Nonetheless, reduced depression might be predicted compared to a similarly symptomatic control group with unrelated illness. (This prediction is complicated by possible counteracting biochemical effects, if downregulation of serotonin function occurred following selective binding by carbamates to the serotonin-binding site on the ACh receptor; or if catecholamine downregulation occurred. These actions would be expected to act in the reverse direction and might attenuate or reverse positive mood effects resulting from changes in the cholinergic system, depending on which effects were most sensitive to chronic alteration.)

Some PGW veterans report eye and visual function problems. Increased tearing is among the acute effects of PB on the eyes. If cholinergic downregulation is in effect, ill PGW veterans might be expected to have reduced lacrimation, which can be assessed by a Schirmer test, rendering it one of the more cheaply and easily tested predictions.

**Affected Brain Areas.** Brain areas in which regional cerebral blood flow is most enhanced acutely by carbamates (physostigmine, which readily penetrates the brain) should be those brain areas in which regional cerebral blood flow reduction might be expected chronically, measured using quantitative SPECT (a technique that measures regional cerebral blood flow) or functional MRI. (More generally, with a dysregulation rather than a downregulation hypothesis, alterations in timing or degree of blood flow to these areas would be expected.)

Heightened regional cerebral blood flow with physostigmine acutely would be presumed to reflect heightened binding and activity of ACh. By hypothesis, this might coincide with regions in which chronic regional cerebral blood flow

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5Alternatively, there could be increased mood lability and a heightened depressogenic effect of ACh acting agents if low ACh function were accompanied by compensatory muscarinic receptor upregulation; however, such receptor upregulation has primarily been reported for nicotinic receptors.
reductions are seen in ill PGW veterans, corresponding to reductions in ACh activity. Although there are reasons this might not be so (since areas acutely responsive to carbamates may not be those most subject to dysregulation), nonetheless the extent to which this is borne out (or not) adds (or subtracts) support from this hypothesis. These areas are discussed in greater detail in the next point, which looks at the functional correlates of this prediction.

Clinical symptoms in ill PGW veterans should correlate with functions governed by brain (and muscle) regions influenced by carbamates. Functions governed by brain areas most affected by carbamates (with physostigmine as an index) should be selectively affected in ill PGW veterans. (The prior prediction related to blood flow measures of activity across regions in the brain. This relates to functional and clinical correlates of activity in those regions.)

Regional cerebral glucose utilization in rats has been examined following administration of the related carbamate AChE-inhibiting compound physostigmine. Physostigmine, at doses of 0.2 and 0.5 mg/kg in rats, measurably increased local cerebral glucose utilization in 15 and 22 regions, respectively, out of 43 regions studied (Bassant, Jazat, and Lamour, 1993). Involved areas included the magnocellular preoptic nucleus (part of the hypothalamic pituitary axis, which is involved in neuroendocrine regulation), ventral thalamus (involved with the amygdala in conditioning), insular cortex (taste), cingulate cortex (concentration, attention), medial septal area (long-term memory), thalamus (gateway to the cortex), superior colliculus (eye movement control), interpositus nucleus (coordination of movement), and the septo-hippocampal system (episodic memory), but not the parietal cortex (sensory motor transformations) (Bassant, Jazat, and Lamour, 1993; and Sejnowski, 1997, for functions of brain regions). The regional topography of the changes in cerebral blood flow reportedly overlapped the distribution of the M2 muscarinic receptors and that of AChE activity, suggesting that the major effects of physostigmine on cerebral glucose utilization resulted from their anticholinesterase action (Bassant, Jazat, and Lamour, 1993).

Another study noted highly localized increased glucose utilization, in rats given physostigmine, in the superior colliculus (eye movements) and anteroventral thalamus (Dietrich, 1984). In humans, studies of cerebral glucose utilization show physostigmine was retained longest in areas rich in AChE, such as the striatum, compared to areas poor in AChE, such as the cerebral cortex (Pappata, Tavitian, et al., 1996). The cerebral distribution of radiolabeled physostigmine was putamen-caudate > cerebellum > brainstem > thalamus > cerebral cortex. Pending studies with finer levels of resolution, if ACh dysregulation is reflected in altered regional blood flow and if it contributes to illness in PGW veterans, then abnormal SPECT findings might be expected to follow this distribution (with the proviso noted above that areas with greatest acute effect might not be
those most susceptible to dysregulation), predicting particular effect on the caudate-putamen.

If PB is postulated to be a cause of illness, with symptoms mediated by ACh dysregulation, such dysregulation might most likely occur in areas most affected by PB acutely. For predictive purposes, it is desirable to examine effects mediated by highly specific areas known to control specific functions. One such area is the superior colliculus, which featured in both the rat studies. Rat studies indicated that physostigmine concentrates in this region (Dietrich, 1984; Bassant, Jazat, and Lamour, 1993), and studies support similar involvement in humans, since physostigmine corrects errors in antisaccadic eye movements, in subjects with a condition termed “progressive supranuclear palsy” (Blin, Mazetti, et al., 1995). One prediction from the theory of cholinergic dysregulation is that superior colliculus function may be affected in some ill PGW veterans, producing abnormalities in eye movements, which are controlled by superior colliculus activity. This is one potential function for which sensitive tests should be devised. Some evidence suggests that objective measures of eye movement function are in fact significantly affected in some ill PGW veterans (Haley, Hom, et al., 1997), and efforts are ongoing to find more sensitive oculomotor measures.

In humans, positron emission tomography for AChE using radiolabeled 11C-physostigmine tracer (physostigmine again being closely related to PB) showed the following distribution of 11C-physostigmine, typical of AChE activity: putamen-caudate > cerebellum > brainstem > thalamus > cerebral cortex, with a striatal:cortex ratio of 2 (Barker, Loewenstein, et al., 1987). At times more than 20 minutes after the physostigmine injection, regional retention of 11C-physostigmine was consistent with the regional distribution of AChE activity and concentration in postmortem human brains. The average regional brain concentration of radioactivity at 25–35 minutes were as follows:

- Putamen: $2.98\pm0.87$
- Cerebellum: $2.28\pm0.58$
- Brainstem: $1.94\pm0.49$
- Thalamus: $1.74\pm0.40$
- Amygdala: $1.83\pm0.52$
- Hippocampus: $1.70\pm0.40$
- Temporal cortex: BA 22, 21, 20, 38, 28): $1.63\pm0.50$
- Frontal associative cortex (BA 9,10): $1.47\pm0.40$
- Parietal associative cortex (VA 7,40): $1.49\pm0.37$
- White matter: $1.45\pm0.40$. 
This study did not report on fine resolution effects, so impact on the superior colliculus was not separately noted. Because of prominent effects in the putamen-caudate, PGW veterans should be monitored for possible early onset Parkinson’s disease. Note that tremor has been reported following carbaryl exposure (Ray and Poddar, 1985), and early onset Parkinson’s has been related to pesticide exposure, which also includes AChE-inhibiting agents, such as carbamates and OPs (Butterfield, Velanis, et al., 1993; Davis, Yesavage, et al., 1978).

Similarly, in PET studies in baboons, 11C-physostigmine uptake was higher in brain regions with high AChE activity and was prevented by preliminary perfusion by excess unlabeled physostigmine (Tavitian, Pappata, et al., 1993).

If symptoms in some ill PGW veterans result from cholinergic dysregulation, then administration of drugs that enhance or reduce ACh function should affect these symptoms.

Downregulation would be anticipated to produce low cholinergic action. If symptoms result from low ACh action, then ACh enhancing drugs should lead to benefit. This is empirically testable.

Similarly, symptoms known to be alleviated with cholinergic drugs might be expected to be present in ill PGW veterans, if ACh downregulation contributes to symptoms.

Symptoms of low cholinergic action may be helped by cholinergic drugs, and conversely symptoms helped by cholinergic drugs may (sometimes) result from low cholinergic action. (They need not always do so, however.) Some of these symptoms might occur in ill PGW veterans. (If one or two symptoms occur, this evidence by itself remains only suggestive, as ability of a nerve signaling chemical to assist in a function does not necessarily imply that reduced activity of that chemical contributed to the deficit. Presence of multiple such symptoms enhances the likelihood of a cholinergic relation to symptoms in ill PGW veterans. However, if altered regulation of ACh does not fit a simple descriptor of reduced or increased activity, all symptoms may not uniformly respond to agents that simply increase or reduce ACh action.)

**Clinical Uses and Comparison to Symptoms**

Examination of identified clinical uses of ACh-enhancing agents and comparison to symptoms in PGW veterans may thus be instructive.

- Learning and memory: AChE inhibitors physostigmine and tacrine have been shown to help reverse learning and memory deficits in some conditions involving memory disorders, which in some instances are believed to
reflect cholinergic underactivity (Davis and Yamamura, 1978; Christie, Shering, et al., 1981; Murray and Fibiger, 1985) and to enhance memory in normal subjects (Davis, Mohs, et al., 1978). Specific functions enhanced include serial learning (Sitaram and Gillin, 1980), short-term consolidation of low-imagery words (Sitaram and Gillin, 1980), retrieval of clustered information (Sitaram and Gillin, 1980), and long-term memory (Davis, Mohs, et al., 1978). As noted earlier, subjective memory deficits are commonly reported in ill PGW veterans.

- Pyridostigmine has been used to assist in various conditions of fatigue and motor weakness, including myasthenia gravis, some HIV associated fatigue, “neurotic” fatigue (that is, for which no organic cause had been identified), and drop attacks in the elderly (See Chapter Three, “Characteristics of PB,” section on medical uses).

- Nicotine has been used to treat sleep apnea; sleep apnea may occur at increased rates in ill PGW veterans. (See Chapter Fifteen, “Other Considerations”).

- Nicotine has been used to treat diarrhea in ulcerative colitis; diarrhea has been reported at increased rates in ill PGW veterans (See Chapter Fifteen).

- Physostigmine has reduced eye movement errors in subjects with progressive supranuclear palsy, as above (Blin, Mazetti, et al., 1995). Some evidence suggests abnormalities in saccadic eye movements in ill PGW veterans, as noted earlier.

- Physostigmine has led to improvement on other neuropsychological tests in progressive supranuclear palsy, although not in that condition’s motor deficits (Blin, Mazetti, et al., 1995). Some studies suggest that ill PGW veterans may have subtle deficits in neuropsychological tests; whether improvement in function would occur with ACh agents, or would occur more than in controls, is not known.

- Pain has been reduced with ACh agonists (Sitaram and Gillin, 1980) (see Chapter Fifteen for more detail). Pain is common in ill PGW veterans.

- Cortical arousal has been reportedly increased with ACh agonists (Sitaram and Gillin, 1980). Fatigue and reduced concentration and attention have been reported by ill PGW veterans.

**LIMITATIONS OF THE HYPOTHESIS**

“Downregulation” appears to be a common compensatory phenomenon in the face of pharmacologically heightened signaling, as a mechanism to counteract excessive nerve signaling. However, many concerns limit our ability to impute
illnesses in PGW veterans to downregulatory phenomena. Most important, PB is normally excluded from central access, and would be poorly positioned to induce downregulation. (Chapters Six, Seven, and Eight discuss issues related to whether special circumstances may have been present that enhanced effect of PB, or central access of PB.) Second, data suggesting downregulation have been derived largely from animals, often using agents that more readily enter the CNS (particularly in studies looking at central effects), at doses that produce substantial AChE inhibition. Similar effects may or may not occur at lower doses and lesser levels of choline inhibition in humans, using PB in conditions of stress. Third, the time-course of different elements of the compensatory changes has not in all instances been assessed, but many of the changes are substantially reversed in a relatively short time. The time-course for different changes in regulation appears to differ (based on limited available evidence), which could be speculated to lead to loss of the normal coordination of signaling mechanisms. It is not known whether changes could persist or perhaps even evolve as a result of dyscoordination of regulation mechanisms leading to their own compensatory changes, following relatively low doses of PB (with or without other AChE inhibitor coexposures). Fourth, not all changes need be downregulatory—indeed, evidence suggests that opposite changes may occur in the muscarinic and nicotinic receptors, in rat brain, following the same exposure (Nilsson-Haransson, Lai, et al., 1990). On a related note, effects for cholinergic agonists and antagonists may occur differentially in different brain and body sites (Schwab, Costa, et al., 1983). While there is more evidence—clinically and at the tissue level—for downregulation than for other forms of aberrant regulation, the totality of alterations in regulation might most appropriately be termed “dysregulation.”

Complicating the evaluation of this hypothesis, different effects of PB and other AChE inhibitors seem to obtain in different muscle groups and in different central sites in animals—perhaps reflecting such factors as different populations of ACh receptors, and different types of AChE (Kamal and al-Jafari, 1996). These differences are themselves a function of the particular AChE-inhibiting chemical (for reasons that are poorly characterized but presumably reflect differences in “specificity” of each agent). Moreover, regulatory changes in the acetylcholinergic system may differ substantially across species, even relatively close species, such as rats and mice. In mice, “denervation” leads to dramatic increases in ACh receptor–encoding genetic material, whereas muscle denervation has little effect in rats (Goldman, Evans, et al., 1987). Whether there also exist differences from one animal to another within a species has not been evaluated. Such individual differences—perhaps regional differences in susceptibility—might be more prominent in humans, since homogeneous groups of inbred animals reared in similar conditions are typically used to reduce individual variation in animal studies and since flexibility in brain
development becomes more prominent as one “ascends” the phylogenetic spectrum.

In any case, the clinical consequences and time-course of cholinergic dysregulation, and the individual differences in ability to restore normal functioning, remain poorly defined. Evidence is insufficient to conclude that such differences do or do not contribute to illness in some PGW veterans.

OTHER NEUROTRANSMITTER SYSTEMS

As previously noted (Chapter Three), PB affects not only the acetylcholinergic system but also other neurotransmitter systems.

Glutamate

Glutamate is the major excitatory neurotransmitter in the brain. Moreover, ACh presynaptically modulates (enhances) release of glutamate. For this system, another form of “downregulation” or dampening of the transmitter system may obtain—in the form of “excitotoxicity.” Excessive amounts of the intrinsic excitatory amino acid glutamate may excite brain neurons in a manner that results in death of these neurons. Moreover, excitotoxic neurodegeneration may occur with relatively low levels of excitotoxic substances (e.g., glutamate) when neuronal energy metabolism is impaired (Whetsell, 1996). A family of glutamate-sensitive receptors made up of subtypes that are selectively sensitive to a number of different glutamate analogs occur in the mammalian nervous system and respond differently to stimulation. Both “fast” and “slow” (also called “direct” and “indirect,” “acute” and “chronic”) excitotoxicity have been described (Whetsell, 1996).

Whether excitotoxicity of the glutamate system has any reference to illnesses in PGW veterans is unknown. Additional work could seek to define the locations and characteristics of glutamate receptors that are potentially “sensitive” to carbamates—that is, for which increased ACh release (from AChE inhibition, from PB or similar agents) acts on presynaptic receptors of glutamate-releasing neurons to increase release of glutamate, leading to potential for excitotoxicity.

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6Such excitotoxicity is thought by some to be involved in development of a motor-neuron condition termed amyotrophic lateral sclerosis. Both familial and sporadic cases occur, and GLU receptors, GLU metabolism, and/or GLU uptake mechanisms may all play a role (Whetsell, 1996). According to DoD sources, nine cases of amyotrophic lateral sclerosis have occurred among PGW veterans. The Oversight Report of the Subcommittee on Human Resources House Committee on Government Reform and Oversight appears to quote 1.4–1.7 cases as expected, but this is evidently the number expected in a single year, which would seem consistent with current reports of amyotrophic lateral sclerosis (Subcommittee on Human Resources, 1997 672).
Serotonin

Serotonin is believed to be the endogenous ligand (substance that binds), the special carbamate-binding site on nicotinic acetylcholinergic receptors to which physostigmine (and presumably pyridostigmine, if it enters the brain) binds. Physostigmine not only increases ACh by inhibiting AChE but also directly binds to this site, the “galanthamine”-binding site (named after another drug that also binds to this site), thus modulating ACh release. That is, such carbamates essentially mimic the action of serotonin (Albuquerque, Pereira, et al., 1997; Albuquerque, Alkondon, et al., 1997). The opiate codeine also binds to this site. Since the acetylcholinergic system is believed to be involved in pain regulation, as is the opiate system and the serotonergic system—in the case of fibromyalgia—it is justifiable to wonder whether this special binding site on the nicotinic receptor could reflect a common point of action. Further study may clarify whether excessive binding at this site leads to alterations that could contribute to enhanced pain of a character resembling “fibromyalgia,” such as that reported by some PGW veterans.

Dopamine

Since the ACh system modulates dopamine release (see Chapter Three), there may be dysregulation of dopamine—upregulation, downregulation, or a combination—resulting from effects on the cholinergic system.

Catecholamines

The effect of ACh on release of catecholamines is discussed earlier in this chapter. ACh regulates catecholamine secretion from the adrenal medulla. Catecholamines are themselves subject to downregulatory influences (Hardman, Limbird, et al., 1996). Most but perhaps not all of these are of short time-course. It is not known whether low catecholamine function occurs as a result of catecholamine downregulation following delivery of PB. The combination of PB and stress would be expected to lead to a catecholamine surge, possibly producing at least temporary downregulation in the catecholamine system.

MORE COMPLICATIONS

As work continues to unravel potential effects of dysregulation or downregulation of ACh and perhaps of other neurotransmitter systems, the complexity of these systems must always be borne in mind. There exist a multiplicity of types of nicotinic and muscarinic receptors, which may behave
differently. They are expressed differentially in different parts of the brain. A number of distinct choline types are also present, indicated by distinct isoenzyme bands (up to 20 bands have been described in the rat (Nagayama, Akahori, et al., 1996)). Impairments in a given system may be evident on challenge with one agonist but not another (Ghigo, Goffi, et al., 1992). There may be male/female differences in enzyme patterns; such differences have been demonstrated in other mammals (Nagayama, Akahori, et al., 1996). Many elements of regulation could be involved, each with possible individual differences and each potentially influenced by other specific properties of that individual’s nervous system. Certainly native differences in regulation of different neurotransmitter systems have been identified, and such differences may play a role in individual susceptibility to dysregulation. (These differences have been related to functional differences in risk of addiction to selected substances, in harm avoidance, and in susceptibility to aggression, among other factors.)

CONCLUSION

Because the cholinergic system is known to be involved in many important functions, including memory, sleep, pain, and muscular activity, down-regulation or, more generally, dysregulation might be expected to produce abnormalities in these domains—domains reflected prominently in complaints of PGW veterans, for whom memory complaints, sleep disruption, and musculoskeletal complaints (including pain) have—along with rash—headed the list of complaints. (A possible role in the development of skin symptoms is discussed in Chapter Fifteen.) While complaints in these domains could arise from unrelated causes, there are reasons to seriously evaluate the possibility that PB may be a contributing factor. First, many ill PGW veterans were exposed to PB; second, changes resulting from administration of PB and lasting long after cessation of PB have been identified, though these results are primarily based on studies in neuromuscular junctions in animals; and third, changes of this type—if similar changes occurred centrally, arising from enhanced access of PB to the CNS in conditions of stress or chemical combinations, following doses of PB such as those given in PGW veterans—would be plausible causes or contributors to such symptoms as those described by ill veterans. (Musculoskeletal and even CNS problems might arise even without such central access by PB, through peripheral actions on the neuromuscular junction and on catecholamine release.) Nonetheless, the time-course and dosing characteristics of these changes have not been well characterized. It is unknown what magnitude of change in cholinergic function would be required to produce symptoms such as those described by PGW veterans (symptoms that, while important to veterans, do not produce readily
detectable abnormalities on most neuropsychiatric batteries), and it is unknown whether the dose and time-course of the downregulatory effect—or of heightened sensitivity effects, if any—are compatible with such changes. It is possible that effects, which may differ for different brain areas and regulatory components, could interact to produce alterations that evolve over time, though this possibility is speculative.

At the time this report was first circulated (including this chapter on dysregulation), ACh system dysregulation had not been previously proposed as a mechanism to consider in relation to illness in PGW veterans. Since then, additional evidence is emerging that suggests that such dysregulation may occur. More recent findings are included in an Addendum.

**SCIENTIFIC RECOMMENDATIONS**

- Additional research is needed into the effects of AChE inhibition by agents—particularly carbamates—on central and peripheral nicotinic and muscarinic signaling. Such research should include investigation into effects on receptors (receptor density, binding affinity, sensitivity to action of ACh once bound), considering each identified receptor type separately; on nerve innervation (e.g., withdrawal of nerve terminals); production and release of ACh from signaling cells; and breakdown of ACh, including effects if any on AChE production and metabolism. Special attention should be paid to doses required for each effect, influence on each effect of the time-course of carbamate delivery, time-course of the effect (e.g., does it persist, increase, or reverse on discontinuation of the carbamate), regional brain differences in the effect (and their relation, or lack of relation, to specific receptor types that predominate in those brain areas), influence on these outcomes of individual differences in processing of PB and ACh (per Chapter Eight), and the influence of concomitant exposures with such agents as pesticides, nerve agents, cigarettes, and others (as per Chapter Nine). Both baseline “tone” of nicotinic and muscarinic systems, as well as reactivity to stimulation, should be assessed. (Evidence from several domains that low baseline function of a neurotransmitter system in some conditions is associated with high reactivity. This could occur, for instance, if there is reduced neurotransmitter release tonically but increased receptor density or binding affinity.)

- Similar tests should be performed to assess function of other neurotransmitter systems influenced by carbamates and by ACh, including the GABA-ergic, glutamatergic, serotonergic, and dopaminergic as well as catecholaminergic systems.
• Additional efforts should be made to assess, at as fine a scale as possible, baseline and task-specific regional blood flow with and without administration of physostigmine, using quantitative SPECT and functional MRI. (This makes the plausible assumption that, if PB crossed the blood–brain barrier, its regional effects would be similar.) Quantitative SPECT and functional MRI studies should be undertaken in ill PGW veterans and controls to examine regional cerebral blood flow. If abnormalities are seen in ill PGW veterans, the regions of altered distribution should be compared to regions altered with administration of physostigmine.

• Efforts should be made to identify objective tests of function related to brain areas in which physostigmine (a related carbamate that crosses into the brain) has been shown to alter regional glucose utilization. (These areas are discussed above.) Once again, it is reasonable to assume that if PB crosses into the brain, the areas affected will likely be similar to those affected with physostigmine, and areas in which effects of AChE inhibition with PB are greatest (indicated by alterations in glucose utilization) are a priori more likely to be areas in which altered regulation of function would ensue.

• Similar efforts should be made to identify sensitive tests for peripheral deficits that might relate to AChE dysfunction.

• Efforts could be made to institute consented, double-blind treatment trials employing agents that augment cholinergic function, such as nicotine, physostigmine, or antihistamines, which have AChE-inhibiting action and increase central ACh (Dringenberg, De Souza-Silva, et al., 1998; Laine-Cessac, Turcant, et al., 1993) or alternatively with agents that have anticholinergic effects. Such trials could test the hypothesis that symptoms in ill PGW veterans arise as a result of cholinergic dysregulation. Symptoms that might respond to cholinergic augmentation include sleep apnea—from which musculoskeletal pain, fatigue, and cognitive dysfunction might ensue—cognitive complaints, such as problems with memory or concentration; diarrhea; pain; and perhaps rash. (If the theory of cholinergic kindling for chemical sensitivities has merit, anticholinergic agents might be hypothesized to produce benefit for chemical sensitivities. However, most symptoms in ill PGW veterans appear primarily consistent with low cholinergic function.) Tests employing selective nicotinic, muscarinic, and antinicotinic or antimuscarinic agents might help to define whether or how dysregulation of the nicotinic and/or muscarinic systems contributes to symptoms in ill PGW veterans.
SUMMARY ANALYSIS

Exposure

Exposure to PB occurred in an estimated 250,000 to 300,000 veterans (Brake, 1997).

Compatible Symptoms

Symptoms of downregulation or dysregulation of the cholinergic system (which may include elements of depressed and heightened cholinergic responsiveness) would be expected to include problems with memory, learning, sleep, musculoskeletal function, GI motility, and pain. Nicotine treatment has reportedly led to benefits for several of these conditions, including memory and cognitive dysfunction, diarrhea, and sleep apnea. Sleep apnea treatment in turn may lead to resolution of secondarily induced problems, including soft tissue pain (fibromyalgia), fatigue, and memory/cognitive dysfunction. These symptoms are prominent in ill PGW veterans. (Sleep and pain are discussed in greater detail in Chapter Fifteen.)

Link Between Exposure and Symptoms

No direct scientific evidence exists to either support or refute a link between cholinergic dysregulation and symptoms in ill PGW veterans.