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Effect of Repeated Organophosphate Administration on Carbachol-Stimulated Phosphoinositide Hydrolysis in the Rat Brain

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MUNDY, W. R., T. R. WARD, V. F. DULCHINOS AND H. A. TILSON. *Effect of repeated organophosphate administration on carbachol-stimulated phosphoinositide hydrolysis in the rat brain.* PHARMACOL BIOCHEM BEHAV 45(2) 309–314, 1993.—The effects of repeated exposure to two organophosphates on the turnover of phosphoinositides, the second messenger system coupled to the M_1 and M_3 subtypes of muscarinic receptors, were examined in the rat hippocampus. Repeated diisopropylfluorophosphate (DFP) exposure (0.2–0.8 mg/kg, SC) decreased brain acetylcholinesterase activity and muscarinic receptor density. The incorporation of [^{3}H]*myo*inositol into brain slices was also decreased. Phosphoinositide turnover was measured as the accumulation of [^{3}H]inositol phosphates (IP) in the presence of lithium. DFP did not affect basal IP accumulation, but decreased carbachol-stimulated IP accumulation in the hippocampus after 0.4 and 0.8 mg/kg. The effects of repeated disulfoton administration (2.0 mg/kg, IP) were also examined in the hippocampus. Similar to DFP, repeated disulfoton exposure decreased acetylcholinesterase activity, receptor density, and carbachol-stimulated IP accumulation. The incorporation of *myo*inositol, however, was increased in disulfoton-treated rats. These data indicate that repeated organophosphate exposure results in a functional decrease in muscarinic receptor activity, as well as changes in *myo*inositol incorporation into phospholipids.

Organophosphorus compounds Cholinesterase inhibitors Muscarinic receptors Phosphoinositide hydrolysis
Diisopropylfluorophosphate Disulfoton Hippocampus

MOST organophosphorus compounds are potent inhibitors of acetylcholinesterase (AChE), the enzyme primarily responsible for the inactivation of acetylcholine. Exposure to AChE inhibitors results in signs of cholinergic overstimulation, including salivation, lacrimation, miosis, weakness, and tremor. Repeated exposure to cholinesterase inhibitors can result in adaptive changes that significantly attenuate toxicity in the presence of continued reduction in AChE activity (24). The mechanism underlying the development of this tolerance is believed to involve a decrease in or downregulation of cholinergic muscarinic receptors. Consistent with this hypothesis, previous studies have demonstrated downregulation of muscarinic receptors in the brain and other tissues after repeated exposure to a number of organophosphates (5,8–10,25,26).

Recently, the effects of repeated exposure to cholinesterase inhibitors on muscarinic second messenger systems have been studied. An important receptor-linked signal transduction system for the muscarinic receptors in the brain involves the hydrolysis of membrane phosphoinositides (PIs) to diacylglycerol and inositol trisphosphate, both of which may act as second messengers [for review, see (10)]. Acetylcholine (3,14) as well as catecholamines (18) and some excitatory amino acids (20) have been shown to act via this PI signalling system. The hydrolysis of PI can be studied directly in brain slices preincubated with [^{3}H]*myo*inositol by measuring the accumulation of radiolabeled inositol phosphates (IPs) in the presence of lithium (2). Experiments concerning the effects of repeated organophosphate exposure on muscarinic receptor-coupled hydrolysis of PI have been inconsistent. Costa et al. (7) found that repeated exposure to disulfoton decreased carbachol-stimulated PI hydrolysis in rat brain cortical slices, and a similar effect on PI hydrolysis in rat cortical slices was observed after repeated exposure to diisopropylfluorophosphate (DFP) (21). Abdallah and El-Fakahany (1), however, reported no change in carbachol-stimulated PI hydrolysis in the rat cortex, hippocampus, or striatum after repeated DFP administration. Further, Kiefer-Day and El-Fakahany (16) recently reported no change in muscarinic receptor agonist-stimulated PI hydrolysis in dissociated brain cells from mice treated with 9-amino-1,2,3,4-tetrahydroacridine (THA), a cholinesterase

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inhibitor. All of these studies reported marked inhibition of AChE activity and, with the exception of THA (16), varying degrees of muscarinic receptor downregulation.

Because previous work did not study dose-response effects of repeated exposure to cholinesterase inhibitors, the present study examined the dose-related effects of cholinesterase inhibition produced by DFP on several cholinergic parameters, including AChE activity, muscarinic receptor density, and agonist-stimulated PI hydrolysis. The present research extends observations made in neocortical tissue to the hippocampus, another brain region that receives cholinergic innervation from the basal forebrain. Effects seen with DFP in the hippocampus were then compared to those produced by another cholinesterase inhibitor, disulfoton. These studies indicate that repeated exposure to two cholinesterase inhibitors at doses that inhibit brain AChE activity and downregulate cholinergic muscarinic receptors attenuate agonist-stimulated PI hydrolysis in the rat brain.

METHOD

Animals and Treatment

Male Long Evans rats (300–350 g) were housed two per cage and provided food and water ad lib. DFP (>95%, Sigma Chemical Co., St. Louis, MO) was dissolved in peanut oil and injected SC 5 days per week for 4 weeks (0.2 and 0.4 mg/kg) or 3 days per week (Mon, Wed, Fri) for 4 weeks (0.8 mg/kg). Exposure to the higher dose 5 days per week resulted in mortality. Control animals received the peanut oil vehicle. In a separate experiment, disulfoton (>98%, Chem Services, Inc., Westchester, PA) was dissolved in corn oil and injected IP at a dose of 2.0 mg/kg daily for 30 days. Control animals received the corn oil vehicle. Animals were killed 24–36 h after the last exposure and the hippocampi removed (13).

Phosphoinositide Hydrolysis

Hydrolysis of PI was studied in hippocampal slices following incorporation of [^{3}H]*myo*inositol by determining accumulation of [^{3}H]IPs in the presence of lithium. Tissue slices (350 × 350 μm) were prepared using a McIlwain tissue chopper and preincubated in Krebs-Ringer bicarbonate buffer (KRB; 118 mM NaCl, 4.7 mM KCl, 0.75 mM $CaCl_2$, 1.18 mM KH_2PO_4 1.18 mM $MgSO_4$, 24.8 mM $NaHCO_3$, 10 mM glucose, pH 7.4) saturated with 95% O_2/5% CO_2 for 30 min in a shaking water bath at 35°C. For labeling, slices were incubated in the presence of 3.2 μM [^{3}H]*myo*inositol (15 Ci/mmol, New England Nuclear, Boston, MA) for 120 min with constant shaking. At the end of the labeling period, the slices were washed with KRB and 50 μl of packed slices were pipetted into separate tubes. Carbachol-stimulated accumulation of [^{3}H]IPs was measured in the presence of 8 mM lithium chloride. Slices were incubated in the presence or absence of the muscarinic receptor agonist for 30 min in a final volume of 250 μl. The reaction was stopped by the addition of 1.0 ml of a chloroform/methanol/HCl (100 : 200:2) mixture. [^{3}H]IPs were separated and measured according to the method of Berridge et al. (2), as modified by Tandon et al. (30). Total IP accumulation was calculated as dpm aqueous/(dpm aqueous + dpm organic) for each tube to account for differences in the volume of slices used or the degree of incorporation into phospholipids. Data are expressed as the percentage accumulation above basal (i.e., accumulation in the absence of agonist).

For determination of incorporation, 450 μl ice-cold KRB were added to 50 μl of slices at the end of the 120-min labeling period to stop the reaction. The slices were sonicated and 250 μl was taken for the estimation of protein. The other 250 μl was used for the estimation of incorporation of [^{3}H]*myo*inositol into phospholipids after extraction and separation of phospholipids from the slices (30). Data are expressed as dpm [^{3}H]*myo*inositol incorporated/mg protein during the 120-min labeling period.

[^{3}H]Quinuclidinyl Benzilate (QNB) Binding

Receptor binding was assayed by the method of Yamamura and Snyder (31) as described previously (4). Briefly, tissue samples were homogenized in phosphate buffer (10 mg/ml) and an aliquot taken for AChE activity determination. The remainder was centrifuged at 27,000 × g for 20 min and the resulting pellet was resuspended to a concentration of 0.6 mg protein/ml. Tissues were incubated for 60 min at room temperature with [^{3}H]QNB (32.9 Ci/mmol, New England Nuclear) at eight concentrations ranging from 0.1–2.9 nM. The reaction was terminated by rapid filtration through glass-fiber filters. Nonspecific binding was determined in the presence of 10 μM atropine sulfate. Binding data were calculated relative to protein concentration and binding constants (B_{max} and K_d) were estimated by Scatchard analysis.

AChE Activity

AChE activity in homogenates was determined radiometrically (15) using [^{3}H]acetylcholine iodide (90 mCi/mmol, New England Nuclear) as the substrate (final concentration 0.13 mM). Activity was expressed as nmol acetylcholine hydrolyzed/min/mg protein.

Protein

Protein was determined using the Bradford assay as modified in a commercially available kit (Bio-Rad, Richmond, CA) with bovine serum albumin as the standard.

Statistics

For AChE activity, receptor binding, and [^{3}H]*myo*inositol incorporation data, significant treatment effects for each dose were determined using a one-way analysis of variance (ANOVA). For IP accumulation data, differences in the response of slices from control animals to carbachol were compared using a two-way ANOVA with experiment as one factor and carbachol concentration as the other. Because there was a significant experiment effect, a two-way ANOVA with treatment as one factor and concentration of carbachol as the other was performed for each dose level of DFP and for disulfoton. The accepted level of significance was set at $p \leq 0.05$.

RESULTS

Repeated daily exposure to 0.2 mg/kg DFP caused no observable signs of cholinergic overstimulation. After the second, third, and fourth injections of 0.4 and 0.8 mg/kg DFP, however, animals exhibited tremor, salivation, and diarrhea. These signs gradually diminished and were no longer observed after the second week of treatment. The inhibition of hippocampal AChE activity is shown in Fig. 1 (top). Repeated exposure to DFP resulted in the inhibition of AChE activity of 70–85%. Scatchard analysis of saturation binding experiments with [^{3}H]QNB indicated that repeated DFP exposure significantly decreased receptor density 15–35% in the hippocampus

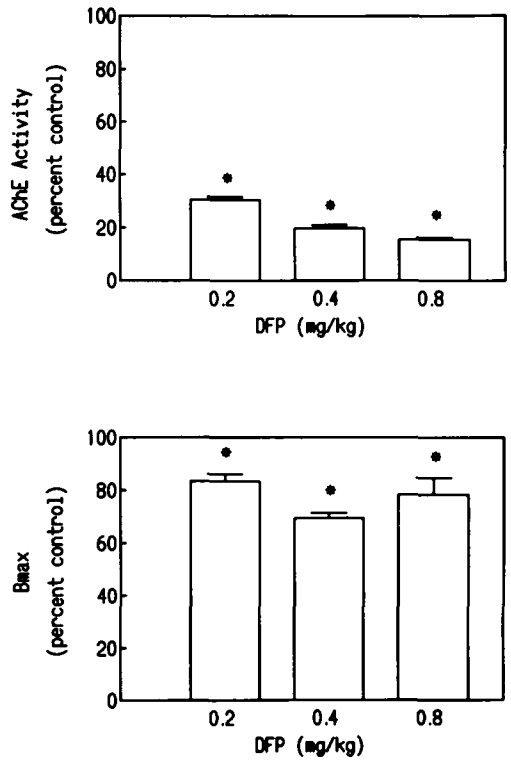

FIG. 1. Effect of repeated diisopropylfluorophosphate (DFP) exposure on acetylcholinesterase (AChE) activity (top) and [^{3}H]quinuclidinyl benzilate (QNB) binding (bottom) in the hippocampus. Rats received DFP or vehicle for 4 weeks and were sacrificed 24–36 h after the last treatment with DFP. All measures were performed in triplicate and data represent the mean (± SE) for 8–10 rats per dose level. Control value for hippocampal AChE activity = 24.6 ± 1.0 nmol acetylcholine hydrolyzed/min/mg protein. Scatchard analysis of the [^{3}H]QNB binding data was used to determine the B_{max} (maximal binding sites) and K_d (apparent dissociation constant). Control B_{max} = 1.57 ± 0.05 pmol/mg protein. DFP exposure did not significantly affect the K_d at any dose (control K_d = 0.48 ± 0.01 nM). *$p \leq 0.05$ compared to control, one-way ANOVA.

(Fig. 1, bottom). There was no effect on affinity (K_d) of receptor sites at any dose (data not shown).

The effects of DFP on the incorporation of [^{3}H]*myo*inositol and basal accumulation of [^{3}H]IPs (in the absence of agonist) are summarized in Table 1. There was an apparent increase in [^{3}H]*myo*inositol incorporation in the hippocampus of animals exposed to 0.2 mg/kg DFP that was not statistically significant. There was a significant decrease in incorporation of [^{3}H]*myo*inositol in the hippocampus from animals exposed to 0.4 and 0.8 mg/kg DFP. Basal accumulation of IPs (adjusted for incorporation), however, was not affected by DFP exposure. The effects of repeated DFP administration on carbachol-stimulated IP accumulation are shown in Fig. 2. Carbachol resulted in a concentration-dependent stimulation of IP accumulation in the hippocampus. In animals receiving 0.2 mg/kg DFP, results were variable and there was no significant effect on IP accumulation. There was a significant decrease in IP accumulation in hippocampal slices from animals receiving 0.4 or 0.8 mg/kg DFP.

Daily exposure to disulfoton resulted in signs of cholinergic overstimulation including tremor during the first week of

TABLE 1

EFFECT OF REPEATED DFP EXPOSURE ON INCORPORATION OF [^{3}H]*MYO*INOSITOL AND BASAL ACCUMULATION OF [^{3}H]IPs IN HIPPOCAMPAL SLICES

DFP (mg/kg)	Incorporation (dpm/mg protein)	Basal IP Accumulation (% incorporation)
0	254,996 ± 13,213	10.8 ± 1.3
0.2	332,542 ± 33,719	11.8 ± 0.9
0	219,684 ± 10,674	9.2 ± 0.3
0.4	175,325 ± 5,332* (−20%)	8.5 ± 0.2
0	252,040 ± 25,115	9.2 ± 1.4
0.8	143,135 ± 14,470* (−44%)	10.0 ± 2.0

Animals were sacrificed 24–36 h after the last treatment with DFP. Basal accumulation of [^{3}H]IPs was measured in the absence of any added agonist. Results represent the mean (±SE) of four to six experiments. Values in parentheses are percent change from control.

*$p \leq 0.05$, significant effect of DFP (ANOVA).

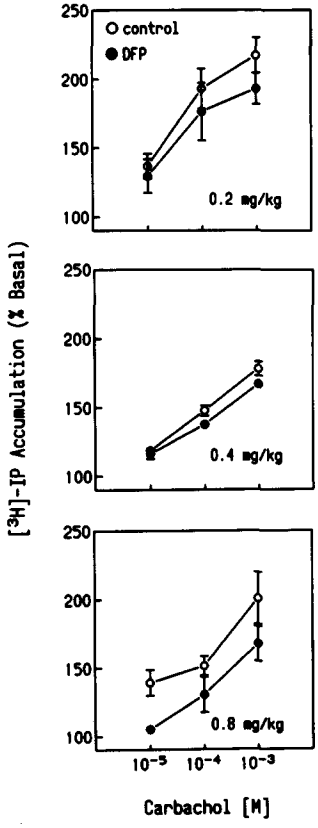

FIG. 2. Carbachol-stimulated [^{3}H]inositol phosphate (IP) accumulation in hippocampal slices from control and diisopropylfluorophosphate (DFP)-treated rats. Data represent the mean (± SE) for four experiments performed in triplicate for each dose level of DFP. Two-way ANOVA indicated a significant effect of carbachol concentration and DFP exposure (with no interaction) after 0.4 and 0.8 mg/kg DFP ($p \leq 0.05$).

exposure, which disappeared during the second week of exposure. In the hippocampus, repeated disulfoton exposure decreased AChE activity by 72% and muscarinic receptor number by 16%; muscarinic receptor affinity (K_d) was not affected (Table 2). The incorporation of [^{3}H]*myo*inositol was significantly increased while basal accumulation of IPs was not affected by disulfoton exposure. Carbachol-stimulated IP accumulation was significantly decreased in animals treated with disulfoton (Table 2).

DISCUSSION

Data from the present experiments extend the results of Costa et al. (7) and Pintor et al. (21) indicating that muscarinic receptor downregulation following prolonged cholinesterase inhibition is associated with a decrease in carbachol-stimulated IP accumulation in rats. These results support the hypothesis that a functional decrease in cholinergic receptor activity is related to the development of tolerance after chronic cholinesterase inhibition. However, several recent studies demonstrated a lack of correlation between receptor downregulation and the development of tolerance (6,27). Thus, other mechanisms, including the uncoupling of receptors from their effector systems, may be involved in the development of tolerance.

Repeated administration of DFP (0.2–0.8 mg/kg) inhibited AChE activity 70–85% in the hippocampus. It should be noted that AChE measurements were made 24–36 h after the last dose of DFP and that some recovery of AChE activity may have occurred during this interval. Thus, the maximal inhibition produced by DFP may be somewhat greater than that reported. In the same animals, there was also a significant downregulation of the total population of muscarinic receptors using [^{3}H]QNB as the ligand. DFP treatment decreased receptor density 15–35% without affecting receptor affinity. These results are in good agreement with previous studies of receptor downregulation after repeated DFP administration in which QNB was used as the ligand (17,22,23).

In the present study, decreases in muscarinic receptor density in the hippocampus were accompanied by a small but significant decrease in carbachol-stimulated IP accumulation in animals exposed to 0.4 or 0.8 mg/kg DFP. Exposure to 0.2 mg/kg DFP did not result in significant changes in IP accumulation, although the data was more variable. The lack of effect at this dose may be the result of smaller decreases in AChE activity and receptor numbers compared to the higher doses. We also observed a decrease in carbachol-stimulated IP accumulation in the hippocampus that parallels muscarinic receptor downregulation after repeated exposure to disulfoton, another organophosphate cholinesterase inhibitor. These results extend those of previous studies showing similar changes in cortical tissue after repeated administration of disulfoton (7) or DFP (21). The present results, along with those of Costa et al. (7) and Pintor et al. (21), suggest that muscarinic receptor downregulation following repeated administration of organophosphates is accompanied by a functional decrease in receptor activity. These results, however, are in contrast to those of Abdallah and El-Fakahany (1), who found no significant change in PI hydrolysis in the cortex, hippocampus, or striatum after repeated administration of DFP. In addition, they also observed no change in the inhibition of cyclic adenosine monophosphate (cAMP) formation in the same brain regions, a response linked to the M_2 and M_4 receptor subtypes, indicating a lack of correlation between muscarinic receptor downregulation and functional changes in receptor activity. The apparent disparity between the results of Abdallah and El-Fakahany (1) and those described above are probably not due to differences in dosing or in the response of animals to chronic cholinesterase inhibition because

TABLE 2

EFFECT OF REPEATED DISULFOTON EXPOSURE ON AChE ACTIVITY, MUSCARINIC RECEPTOR BINDING, AND PI HYDROLYSIS IN HIPPOCAMPAL SLICES

	Control	Disulfoton	*n*
AChE activity (nmol/min/mg protein)	31.0 ± 1.6	8.7 ± 0.7* (−72%)	4
[^{3}H]QNB binding			
B_{max} (pmol/mg protein)	1.85 ± 0.02	1.56 ± 0.10* (−16%)	4
K_d (nM)	0.030 ± 0.002	0.029 ± 0.001	4
Incorporation (dpm/mg protein)	202,556 ± 11,444	271,917 ± 12,610* (+34%)	6
Basal IP accumulation (% incorporation)	10.1 ± 0.9	11.5 ± 0.7	3
Carbachol-stimulated IP accumulation (% basal)			
10^{-3} M	180 ± 18	162 ± 4*	3
10^{-4} M	143 ± 2	125 ± 5*	3
10^{-5} M	127 ± 4	111 ± 1*	3

Animals were sacrificed 24–36 h after the last treatment with disulfoton (2 mg/kg). Accumulation of [^{3}H]IPs was measured in the absence (basal) and presence of the muscarinic agonist carbachol. Results are the mean (± SE) of the number of experiments indicated by *n*. Values in parentheses are percent change from control.

*$p \leq 0.05$, significant main effect of disulfoton (ANOVA).

receptor number (measured using QNB) was decreased 22–44%, a reduction similar to that obtained in the studies described above. In contrast to the reports from other laboratories, Abdallah and El-Fakahany (1) examined PI hydrolysis and cAMP formation in dissociated cell aggregates. It is possible that the manipulations used to produce the cell aggregates affect cell surface receptors so that their response is altered compared to the brain miniprisms used in the previous studies of PI hydrolysis (7,21) and in the present study. It should be noted that the second messenger responses in DFP-treated animals in the study of Abdallah and El-Fakahany (1) are in the same direction and differ only in magnitude from the studies described above. Nonsignificant decreases in IP accumulation were observed in the hippocampus and striatum after repeated DFP exposure. Direct comparison of the different methodologies would be useful in resolving this conflict.

A novel finding in the present study is that organophosphate administration appeared to alter in vitro incorporation of *myo*inositol into phospholipids. DFP exposure decreased incorporation up to 55% while disulfoton exposure increased incorporation by 34%. Administration of 0.2 mg/kg DFP appeared to increase incorporation, although because of the large variability the difference was not significant. These results appear to be in contrast to those of Costa et al. (7), who reported no change in incorporation after repeated disulfoton administration. A major difference between the present study and Costa et al. (7) is the concentration of *myo*inositol available for incorporation during the labeling period. Costa et al. (7) used 0.3 μM [^{3}H]*myo*inositol, an amount typical for studies examining IP accumulation in the presence of lithium. However, the enzyme responsible for the initial step in the synthesis of PI from *myo*inositol, CDP-DG:*myo*inositol transferase, has a K_m in the low millimolar range (12). In addition, concentrations of *myo*inositol are 150–175 μM in the cerebrospinal fluid (29). Thus, the concentration of *myo*inositol typically used to study incorporation in vitro is low and may not accurately reflect the normal physiological process. In the present study, we used [^{3}H]*myo*inositol at a concentration of 3.2 μM and obtained incorporation of approximately 250,000 dpm/mg protein in slices from control animals. This is substantially higher than that obtained by Costa et al. (7). While the concentration of [^{3}H]*myo*inositol used in the present study is still low compared to physiological levels, it may be sufficient to uncover changes in incorporation produced by organophosphates. Experiments examining the effect of varying concentrations of *myo*inositol on incorporation into brain slices will be necessary to address this issue.

The effect of organophosphate exposure on incorporation may be related to the inhibition of AChE and resulting increase in synaptic levels of acetylcholine. Cholinergic agonist stimulation during the incorporation period has been shown to increase the incorporation of *myo*inositol into phospholipids (28). However, this is unlikely in the present experiment because basal accumulation of IPs was unchanged in animals exposed to organophosphates. In addition, DFP and disulfoton appeared to have opposite effects on the incorporation of [^{3}H]*myo*inositol, arguing against a common mechanism of action for the two compounds. It is possible that the organophosphates are acting directly on the enzymes involved in *myo*inositol incorporation into phospholipids. Although DFP has been reported to interfere with phospholipid synthesis (19), the effects of disulfoton on phospholipid metabolism have not been studied. Further research comparing the effects of organophosphates with their oxygen analogs on *myo*inositol incorporation and PI synthesis after both in vivo and in vitro exposure are necessary to understand the mechanism of action of these compounds.

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REFERENCES

1. Abdallah, E. A. M.; El-Fakahany, E. E. Lack of desensitization of muscarinic receptor-mediated second messenger signals in rat brain upon acute and chronic inhibition of acetylcholinesterase. J. Biochem. Toxicol. 6:261–268; 1991.
2. Berridge, M. J.; Downes, C. P.; Hanley, M. R. Lithium amplifies agonist-dependent phosphatidylinositol responses in brain and salivary glands. Biochem. J. 206:587–595; 1982.
3. Brown, E.; Kendall, D. A.; Nahorski, S. R. Inositol phospholipid hydrolysis in rat cerebral cortical slices: I. Receptor characterization. J. Neurochem. 42:1379–1387; 1984.
4. Bushnell, P. J.; Padilla, S. S.; Ward, T.; Pope, C. N.; Olszyk, V. B. Behavioral and neurochemical changes in rats dosed repeatedly with diisopropylfluorophosphate. J. Pharmacol. Exp. Ther. 256: 741–750; 1991.
5. Churchill, L.; Pazdernik, T. L.; Samson, F.; Nelson, S. R. Topographical distribution of down-regulated muscarinic receptors in rat brains after repeated exposure to diisopropylphosphonofluoridate. Neuroscience 11:463–472; 1984.
6. Collins, A. C.; Smolen, T. N.; Smolen, A.; Medhurst, L. J. Cross-tolerance between muscarinic agonists: Role of muscarinic receptors. Pharmacol. Biochem. Behav. 26:173–182; 1987.
7. Costa, L. G.; Kaylor, G.; Murphy, S. D. Carbachol- and norepinephrine-stimulated phosphoinositide metabolism in rat brain: Effect of chronic cholinesterase inhibition. J. Pharmacol. Exp. Ther. 239:32–37; 1986.
8. Costa, L. G.; Murphy, S. D. Passive avoidance retention in mice tolerant to the organophosphorus insecticide disulfoton. Toxicol. Appl. Pharmacol. 65:451–458; 1982.
9. Costa, L. G.; Schwab, B. W.; Murphy, S. D. Differential alterations of cholinergic muscarinic receptors during chronic and acute tolerance to organophosphorus insecticides. Biochem. Pharmacol. 31:3407–3417; 1982.
10. Ehlert, F. J.; Kokka, N.; Fairhurst, A. S. Altered [^{3}H]quinuclidinyl benzilate binding in the striatum of rats following chronic cholinesterase inhibition with diisopropylfluorophosphate. Mol. Pharmacol. 17:24–30; 1980.
11. Fisher, S. K.; Agranoff, B. W. Receptor activation and inositol lipid hydrolysis in neural tissues. J. Neurochem. 48:999–1017; 1987.
12. Ghalayini, A.; Eichberg, J. Purification of phosphatidylinositol synthetase from rat brain by CDP-diacylglycerol affinity chromatography and properties of the purified enzyme. J. Neurochem. 44:175–182; 1985.
13. Glowinski, J.; Iversen, L. L. Regional studies of catecholamines in the rat brain. I. The disposition of ^{3}H-norepinephrine and ^{3}H-DOPA in various regions of the brain. J. Neurochem. 13:655–699; 1966.
14. Gonzales, R. A.; Crews, F. T. Characterization of the cholinergic stimulation of phosphoinositide hydrolysis in rat brain slices. J. Neurosci. 4:3120–3127; 1984.
15. Johnson, C. D.; Russell, R. L. A rapid, simple radiometric assay

for cholinesterase, suitable for multiple determinations. Anal. Biochem. 64:229–238; 1975.
16. Kiefer-Day, J. S.; El-Fakahany, E. E. Muscarinic receptor function and acetylcholinesterase activity after chronic administration of tacrine to mice at therapeutic drug concentrations. Pharmacology 44:71–80; 1992.
17. Lim, D. K.; Hoskins, B.; Ho., I. K. Evidence for the involvement of presynaptic cholinergic functions in tolerance to diisopropylfluorophosphate. Toxicol. Appl. Pharmacol. 90:465–476; 1987.
18. Minneman, K. P.; Johnson, R. D. Characterization of alpha-1 adrenergic receptors linked to [^{3}H]inositol metabolism in rat cerebral cortex. J. Pharmacol. Exp. Ther. 230:317–323; 1984.
19. Nelson, W. L.; Barnum, C. P. The effect of diisopropylphosphorofluoridate (DFP) on mouse brain phosphorus metabolism. J. Neurochem. 6:43–50; 1960.
20. Nicoletti, F.; Wroblewski, J. T.; Novelli, A.; Alho, H.; Guidotti, A.; Costa, E. The activation of inositol phospholipid metabolism as a signal-transducing system for excitatory amino acids in primary cultures of cerebellar granule cells. J. Neurosci. 6:1905–1911; 1986.
21. Pintor, A.; Fortuna, S.; Nalepa, I.; Michalek, H. Effects of diisopropyl fluorophosphate on muscarinic-M_1-receptors and on receptor-mediated responsiveness of the phosphatidyl-inositol system in the cerebral cortex of rats. Neurotoxicology 13:289–294; 1992.
22. Pintor, A.; Fortuna, S.; Volpe, M. T.; Michalek, H. Muscarinic receptor plasticity in the brain of senescent rats: downregulation after repeated administration of diisopropylfluorophosphate. Life Sci. 42:2113–2121; 1988.
23. Raffaele, K.; Olton, D.; Annau, Z. Repeated exposure to diisopropylfluorophosphate (DFP) produces increased sensitivity to cholinergic antagonists in discrimination retention and reversal. Psychopharmacology (Berl.) 100:267–274; 1990.
24. Russell, R. W.; Overstreet, D. H. Mechanisms underlying sensitivity to organophosphorus anticholinesterase compounds. Prog. Neurobiol. 28:97–131; 1987.
25. Sivam, S. P.; Norris, J. C.; Lim, D. K.; Hoskins, B.; Ho, I. K. Effects of acute and chronic cholinesterase inhibition with diisopropylfluorophosphate on muscarinic, dopamine, and GABA receptors of the rat striatum. J. Neurochem. 40:1414–1422; 1983.
26. Smit, M. F.; Ehlert, F. J.; Yamamura, S.; Roeske, W. R.; Yamamura, I. H. Differential regulation of muscarinic agonist binding sites following chronic cholinesterase inhibition. Eur. J. Pharmacol. 66:379–380; 1980.
27. Smolen, T. N.; Smolen, A.; Collins, A. C. Dissociation of decreased numbers of muscarinic receptors from tolerance to DFP. Pharmacol. Biochem. Behav. 25:1293–1301; 1986.
28. Stephens, L. R.; Logan, S. D. Formation of [^{3}H]inositol metabolites in rat hippocampal formation slices prelabelled with [^{3}H]inositol and stimulated with carbachol. J. Neurochem. 52: 713–721; 1989.
29. Swahn, C. G. Gas chromatographic-mass spectrometric determination of myo-inositol in human cerebrospinal fluid. J. Neurochem. 45:331–334; 1985.
30. Tandon, P.; Ali, S.; Bonner, M.; Tilson, H. A. Characterization of receptor-coupled phosphoinositide hydrolysis in the rat hippocampus after intradentate colchicine. J. Neurochem. 53:1117–1125; 1989.
31. Yamamura, H. I.; Snyder, S. H. Muscarinic cholinergic binding in rat brain. Proc. Natl. Acad. Sci. USA 71:1725–1729; 1974.