Ergoline Congeners as Potential Inhibitors of Prolactin Release. 2†,1

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In our attempts to elucidate the prolactin release inhibiting pharmacophore within the ergoline structure, we have prepared one indolealkylamine and several 2-aminotetralin derivatives. These congeners have been evaluated for inhibition of prolactin release in vivo. One congener, 5,8-dihydroxy-2-dimethylaminotetralin, and the drug M-7 significantly inhibited prolactin secretion.

A large variety of ergoline (1) derived compounds are capable of inhibiting the release of prolactin from the anterior pituitary in mammals.[2,3] The antiimplantive,[4,5] antilactation,[6,7] and tumor regression effects[8,9] reported for the various ergoline derivatives can be attributed to their ability to prevent prolactin release. Examples of two such ergoline derivatives include lysergic acid diethylamide[10] (2) and D-6-methyl-8-cyanomethylergoline[4,11] (3).

In our attempts to elucidate the prolactin release inhibiting pharmacophore within the ergoline structure,[1] we have prepared one β-phenethylamine 4, one indolealkylamine 5, and several 2-aminotetralin derivatives 6–13. These congeners have been evaluated for inhibition of prolactin release in an in vivo assay procedure.

Floss et al.[3] have suggested that an intact ergoline ring system is necessary for prolactin release inhibiting activity. However, the increasing bulk of evidence which indicates that inhibition of prolactin release is caused by dopamine agonists[12,13] and the evidence which demonstrates that various ergoline derived compounds have potent dopaminergic agonist effects[14-16] suggest that the ergoline derivatives are able to inhibit prolactin secretion by virtue of their dopamine agonist properties. It seems unlikely that a molecule as large as the entire ergoline ring system should be needed to mimic the action of dopamine if these drugs are indeed interacting with the same receptor. Our approach to congeners of the ergoline structure is based on the expectation that simple molecules, which have certain structural features in common with the ergoline structure, can show biological activity which is similar to that of the ergoline derived compounds.[1]

Although Bach et al.[17] have demonstrated that a substituent at the C-8 position of the ergoline structure is not essential for a potent inhibitory action on prolactin release, some of the compounds which we have prepared have functional groups which can correlate with the C-8 substituent in an active ergoline compound. Thus, the diethylamide moiety in 4 correlates with the diethylamide moiety in LSD (2). The cyanomethylene function in 5, 6, and 10 correlates with the cyanomethyl substituent in 3.

Since the lysergic acid nucleus has been shown to have a high calculated energy of the highest occupied molecular orbital (HOMO),[18] and that substituents on the aromatic ring can increase the energy of the HOMO in amphetamines,[19] our congeners may better mimic the ergoline structure if they contain substituents which have been shown to electronically activate an aromatic nucleus. Therefore, a number of our congeners bear substituents which activate the aromatic ring.

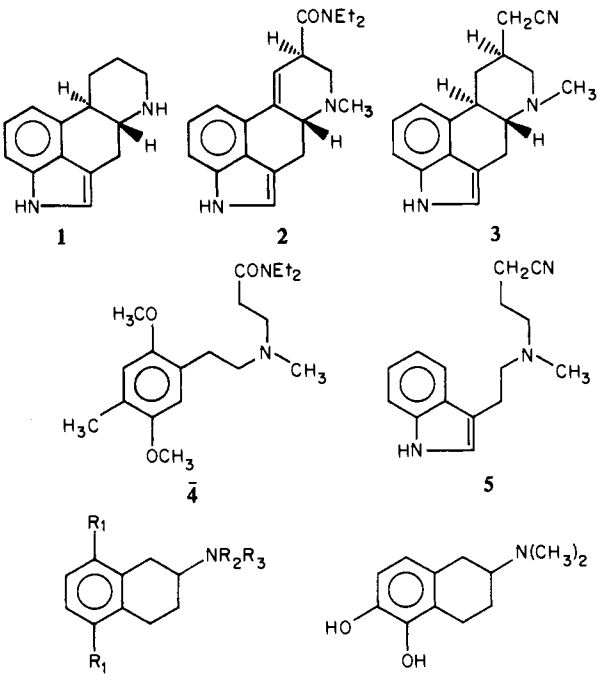
Chemistry. The preparations of 4 and 6 have been previously reported.[1] The preparation of the indolealkylamine 5 is shown in Scheme I. Following the procedure of Speeter and Anthony[20] the tryptamine system was reached by a $LiAlH_4$ reduction of a substituted 3-indoleglyoxylamide 16. The benzylmethyltryptamine so formed was not isolated but immediately debenzylated to *N*-methyltryptamine (17).

The preparations of compounds 8–10 are shown in Scheme II. The synthesis of 18 has been previously reported as an intermediate in the preparation of 7.[21]

Conversion of compounds 7–9 to the dihydroxy derivatives 11–13 was conducted according to Scheme III. Interestingly, the HBr salt of 12 crystallized with 1 equiv of ethanol. This ethanol of crystallization was not removed by heating the salt to 80° at 0.5 mmHg for several hours. Its presence was indicated by elemental analyses and verified by NMR. The salt containing the ethanol of crystallization was used as such in the biological evaluation.

Experimental Section

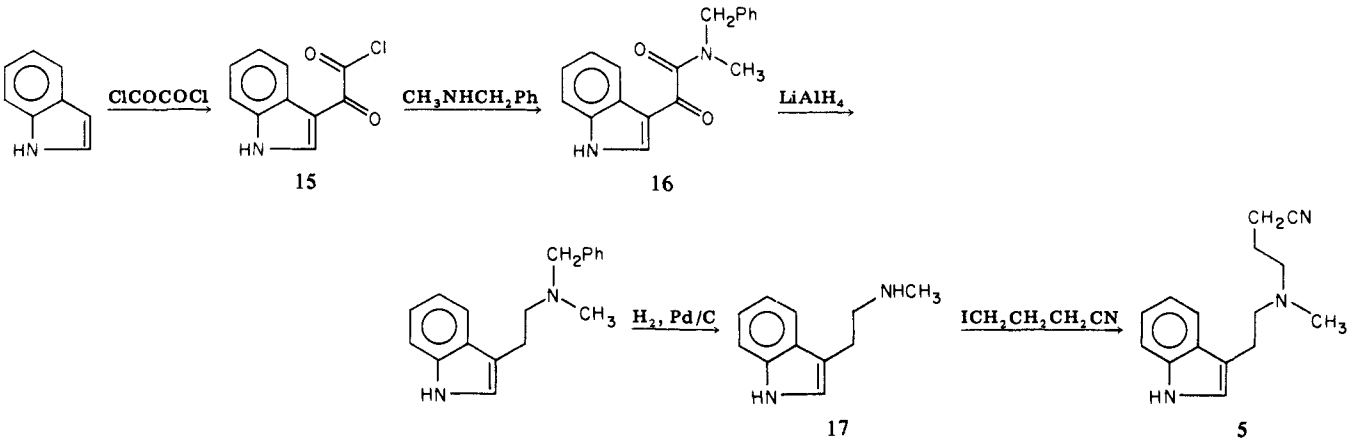
All boiling points are uncorrected. Melting points were determined in open glass capillaries using a Thomas-Hoover Uni-Melt apparatus and are uncorrected. Elemental analyses were performed by Midwest Microlab Ltd., Indianapolis, Ind., and by



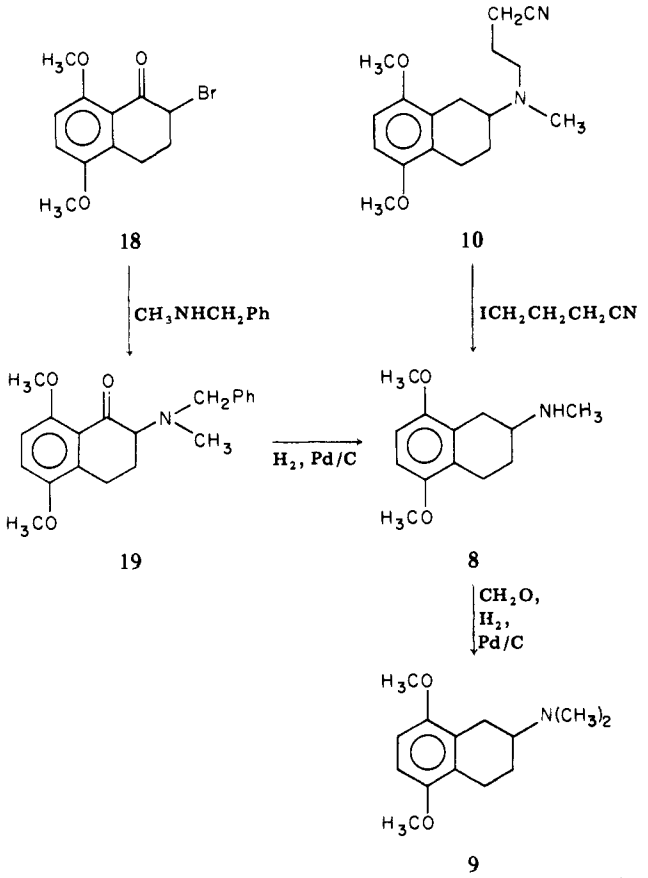
6, $R_1 = H$; $R_2 = CH_3$; $R_3 = CH_2CH_2CH_2CN$
7, $R_1 = OCH_3$; R_2, $R_3 = H$
8, $R_1 = OCH_3$; $R_2 = H$; $R_3 = CH_3$
9, $R_1 = OCH_3$; R_2, $R_3 = CH_3$
10, $R_1 = OCH_3$; $R_2 = CH_3$; $R_3 = CH_2CH_2CH_2CN$
11, $R_1 = OH$; R_2, $R_3 = H$
12, $R_1 = OH$; $R_2 = H$; $R_3 = CH_3$
13, $R_1 = OH$; R_2, $R_3 = CH_3$

† This paper is dedicated to Professor Edward E. Smissman in appreciation of the education, inspiration, and fellowship given to C.F.B.

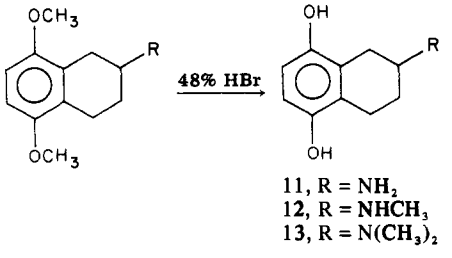
Scheme I



Scheme II



Scheme III



the Division of Medicinal Chemistry and Natural Products, University of Iowa. Where analyses are indicated by symbols of the elements, the analytical results obtained were within ±0.4% of the theoretical values. Infrared spectra were recorded on a Beckman IR-10 spectrophotometer. Nuclear magnetic resonance spectra were recorded on a Varian Associates T-60 spectrometer using tetramethylsilane as an internal standard.

N-**Methyl-*N*-benzyl-3-indoleglyoxylamide (16).** To a solution of 30 g (0.256 mol) of indole in 300 ml of dry ether was added 43.5 ml (0.512 mol) of oxalyl chloride in 100 ml of dry ether dropwise with stirring at a rapid rate, but maintaining the temperature below 10°. After addition was complete the mixture was stirred for 45 min. Filtration provided 52 g (98%) of the crude 3-indoleglyoxalyl chloride **15** as a yellow solid. To a suspension of 41.4 g (0.2 mol) of the crude **15** in 400 ml of ether cooled in an ice bath was added dropwise with stirring 49 g (0.4 mol) of benzylmethylamine in 50 ml of ether. When addition was complete the thick sludge was stirred at room temperature for 3 hr and then filtered. The filtered solid was digested in water and filtered again. The residual solid was allowed to dry and then recrystallized from 1:1 methanol–acetone: yield 41.8 g (71.5%); mp 175–177°. Anal. ($C_{18}H_{16}N_2O_2$) C, H, N.

N-**Methyltryptamine (17).** To a suspension of 12 g (0.31 mol) of $LiAlH_4$ in 400 ml of dry ether with ice bath cooling and vigorous stirring was added portionwise 14 g (0.05 mol) of **16**. The mixture was heated to reflux for 2 hr and then stirred overnight at room temperature. The reaction mixture was decomposed by carefully adding 12 ml of H_2O, 12 ml of 15% NaOH, and then 36 ml of H_2O. The solution was filtered and the solvent evaporated. The crude *N*-methyl-*N*-benzyltryptamine was dissolved in 65 ml of ethanol and hydrogenated over 1 g of 5% Pd/C with several drops of concentrated HCl at 50 psig at room temperature. After 7 hr the catalyst was removed by filtration and the solvent evaporated. The crude product weighed 7.5 g (89%). The residue was taken up in ether and hexane from which it crystallized in the freezer: crystallized yield 3.0 g (36%); mp 80–84° (lit.[22] 85–87°); mp picrate 191–193° (lit.[22] 186–188°).

N-**Methyl-*N*-(3-cyanopropyl)tryptamine Hydrobromide (5).** A mixture of 9.4 g (0.054 mol) of **17** and 5.2 g (0.027 mol) of 4-iodobutyronitrile[1] in 100 ml of benzene was heated to reflux for 18 hr. The reaction was cooled, 50 ml of dry ether was added, and the liquid phase was decanted from the by-product amine salt. The solution was washed once with H_2O and dried ($MgSO_4$), and the product was precipitated with an HBr–ether solution. The oily salt was separated from the ether layer and dissolved in water, the aqueous solution was made basic (NaOH), and the product amine was extracted with ether. The combined ether layers were dried (Na_2SO_4) and concentrated to give the oily product: yield 3.3 g (51%); ir CN stretch, 2250 cm^{-1}. The oily product was passed over a silica column eluted with 4:1 benzene–ethanol. The purified fractions were combined, the solvent was evaporated, the residue was taken up in ether, and the HBr salt was precipitated with an HBr–ether solution. The salt finally crystallized from 1-butanol–ether. Seed crystals induced the crude salt to crystallize without chromatographic purification: mp 121–123°. Anal. ($C_{15}H_{20}N_3Br$) C, H, N.

N-**Methyl-*N*-benzyl-5,8-dimethoxy-2-amino-1-tetralone Hydrochloride (19).** To a solution of 22 g (0.077 mol) of **18**[1] in 200 ml of benzene at 0° under nitrogen was added dropwise with stirring 19 g (0.154 mol) of benzylmethylamine in 100 ml of benzene. When addition was complete the mixture was heated to reflux for 20 hr. The mixture was cooled to room temperature; 300 ml of dry ether was added and then cooled further to 0°. Benzylmethylamine hydrobromide was removed by filtration (76%) and the filtrate extracted twice with 150 ml of 1 *M* HCl. The organic layer was discarded and the acid solution was

evaporated to dryness. The residue crystallized from acetone–ether: yield 9.2 g (33%); mp 155–158°. Anal. ($C_{20}H_{24}NO_3Cl$) C, H, N.

N-**Methyl-5,8-dimethoxy-2-aminotetralin (8).** A mixture of 8.4 g (0.023 mol) of **19** and 1.5 g of 10% Pd/C catalyst in 150 ml of glacial HOAc was hydrogenated at 60 psig and 40° for 23 hr. At this time 5 ml of 72% $HClO_4$ was added and hydrogenation continued for 6 hr more at 45 psig and 50°. The mixture was filtered and KOAc was added to precipitate perchlorate anion. The solution was filtered and evaporated. The residue was dissolved in water, made basic (NaOH), and extracted with ether. The ether was dried (Na_2SO_4) and evaporated. The product was distilled: yield 3.4 g (66%); bp 129–132° (0.2 mmHg). The hydrochloride salt was precipitated with ether–HCl and crystallized from 2-propanol: mp 222–224°. Anal. ($C_{13}H_{20}NO_2Cl$) C, H, N.

N,N-**Dimethyl-5,8-dimethoxy-2-aminotetralin Hydrochloride (9).** A mixture of 0.33 g (0.0013 mol) of **8** hydrochloride was dissolved in water, made basic (NaOH), and extracted into ether. The ether was evaporated. The residue was mixed with 5 ml of 37% CH_2O solution and 0.2 g of 5% Pd/C in 45 ml of EtOH and hydrogenated at 50 psig for 4 hr. The solution was filtered and evaporated. The residue was dissolved in 3 *M* HCl, made basic (NaOH), and extracted with ether. The ether extracts were dried ($MgSO_4$) and filtered and the hydrochloride salt was precipitated with ether–HCl and recrystallized from 2-propanol–ether: yield 0.23 g (67%); mp 207–208°. Anal. ($C_{14}H_{22}NO_2Cl$) C, H, N.

N-**Methyl-*N*-(3-cyanopropyl)-5,8-dimethoxy-2-aminotetralin Hydrobromide (10).** A solution of 3.3 g (0.015 mol) of **8** and 1.44 g (0.0075 mol) of 4-iodobutyronitrile[1] in 50 ml of benzene was heated to reflux for 24 hr. After cooling to room temperature, 100 ml of dry ether was added and the mixture was chilled to 0°. The by-product salt was removed by filtration and the filtrate was washed once with water and dried (Na_2SO_4). The product was precipitated with a fresh ether–HBr solution and recrystallized from acetone–ether: yield 2.2 g (81.5%); mp 156–158.5°. Anal. ($C_{17}H_{25}N_2O_2Br$) C, H, N.

5,8-Dihydroxy-2-aminotetralin Hydrobromide (11). In a two-necked flask fitted with a nitrogen inlet and a reflux condenser 1.0 g (0.0049 mol) of **7** and 15 ml of 48% HBr were heated at 130–140° for 3.5 hr. The mixture was evaporated in vacuo to dryness several times from absolute EtOH solution. The residue was taken up in a small volume of EtOH and diluted with ether whereupon the product crystallized: yield 0.71 g (57%); mp 265–267°. Anal. ($C_{10}H_{14}NO_2Br$) C, H, N.

N-**Methyl-5,8-dihydroxy-2-aminotetralin Hydrobromide (12).** Following the procedure that was used for the preparation of **11**, 0.46 g (0.0018 mol) of **8** was allowed to react with 10 ml of 48% HBr. The product contained ethanol in a 1:1 ratio with the amine: yield 0.41 g (73%); mp 173–175°. Anal. ($C_{13}H_{22}NO_3Br$) C, H, N.

N,N-**Dimethyl-5,8-dihydroxy-2-aminotetralin Hydrobromide (13).** Following the procedure that was used for the preparation of **11**, 0.60 g (0.0022 mol) of **9** was allowed to react with 10 ml of 48% HBr: yield 0.53 g (84%); mp 235–236°. Anal. ($C_{12}H_{18}NO_2Br$) C, H, N.

Pharmacological Evaluation. The target compounds were evaluated for inhibition of prolactin release in an in vivo assay procedure.[2] Briefly, the assay involves ip administration of a 1-mg dose of the test compound to reserpinized male rats followed by radioimmunoassay of serum prolactin content 1 hr after injection. Prolactin was assayed using the kit distributed by the NIAMDD. The results are expressed as nanograms of NIAMDD prolactin RP-1 per milliliter of serum and have been converted to percent inhibition relative to the control value. Ten rats were used for each drug and significance was determined using Student's t test.

Results and Discussion

The results of the prolactin release inhibition assay are shown in Table I. The standard drugs ergocornine and apomorphine inhibited prolactin secretion by 60 and 29% at dosages of 10 μg/rat and 1 mg/rat, respectively. The drug, "M-7" (**14**), which has been shown to be a potent dopamine agonist,[2,3] is found to have a strong inhibitory action in this assay, thereby adding further support to the relatively inactive, while **6** produced a 24% inhibition, which has only borderline significance at this dose level, but may exhibit a significant effect at higher doses. In the aminotetralin series **7–10**, methoxy substituents on the aromatic ring did not produce active compounds. However, hydroxy substituents did appear to confer activity to the aminotetralin structure when the 2-amino group was tertiary. The dramatic increase in activity which occurs when going from the secondary amine **12** to the tertiary amine **13** emphasizes the importance of the tertiary amine to activity in these agents. The structural similarity between **13** and **14** strongly suggests that **13** is a dopaminergic agonist as well. We are now involved in the contention that inhibition of prolactin release results from dopaminergic agonism. The test compounds **4** and **6** have previously been shown to inhibit prolactin secretion in vitro when used in high concentrations.[1] In this assay **4** was confirmation of dopaminergic activity in **13** and other compounds.

Table I. Effect of Ergoline Congeners on Prolactin Secretion in Vivo

Test compd	Dose	No. of rats	Serum prolactin levels, ng/ml ± SE	% inhibn rel to control	Significance
			Assay 1		
Control		10	28.6 ± 2.8		
Ergocornine	10 μg	10	11.3 ± 0.5	60	$p < 0.001$
4	1 mg	10	25.6 ± 1.7	10	N.S.
6	1 mg	10	21.6 ± 1.9	24	Borderline
7	1 mg	10	27.8 ± 2.5	3	N.S.
8	1 mg	10	27.2 ± 2.1	5	N.S.
9	1 mg	10	29.6 ± 2.3	−3	N.S.
10	1 mg	10	29.7 ± 3.4	−4	N.S.
14	1 mg	10	9.1 ± 0.3	68	$p < 0.001$
			Assay 2		
Control		10	25.67 ± 3.06		
Apomorphine	1 mg	10	18.3 ± 3.06	29	$p < 0.05$
11	1 mg	10	27.9 ± 3.1	−9	N.S.
12	1 mg	10	30.8 ± 4.6	−19	N.S.
13	1 mg	10	19.38 ± 2.1	25	$p < 0.01$
			Assay 3		
Control		10	34.1 ± 2.3		
5	1 mg	10	30.01 ± 2.47	12	N.S.

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Improved Delivery through Biological Membranes. 1. Synthesis and Properties of 1-Methyl-1,6-dihydropyridine-2-carbaldoxime, a Pro-Drug of *N*-Methylpyridinium-2-carbaldoxime Chloride†,1

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A dihydropyridine–pyridine type redox pro-drug system was developed for delivering quaternary pyridinium salts through biological membranes. As a first application, the dihydropyridine derivative of *N*-methylpyridinium-2-carbaldoxime chloride (2-PAM) was synthesized using a reduction–addition–elimination sequence. The dihydro-2-PAM obtained has all the required properties for an effective transport through lipoidal barriers and it reverts easily back to 2-PAM as a result of a chemical or enzymatic oxidation process.

The movement of molecules through biological barriers requires passage of the molecular species from one aqueous environment to another aqueous compartment via the cell membrane. It is generally accepted that the ability of substances to penetrate cell membranes can be correlated with their relative solubilities in aqueous and nonaqueous solvents. Molecules which have a high affinity for an aqueous environment find it difficult to enter the proposed lipid interior of the membrane. Therefore, ionic species encounter the most difficulty penetrating various biological membranes. The absorption of many weak organic acids and bases is explicable in terms of the diffusion of only the un-ionized form across the lipoidal barrier. However, the ionic quaternary pyridinium and/or ammonium salts, unless they are small, can be absorbed through a biological membrane, such as the gastrointestinal wall, only as a complex with some endogenous substance[2] or as a lipid-soluble ion pair.[3]

The boundary between the plasma and the central nervous system compared to the boundary between the plasma and other tissue organs is very impermeable to a wide variety of water-soluble, lipid-insoluble compounds. This barrier to highly polar species has been termed the blood–brain barrier (BBB). The endothelial cells in the brain capillaries appear to be joined by continuous, tight, intercellular junctions.[4] Therefore, the molecules which cross this barrier must pass through the cells rather than between them. There exist a few areas in the brain where the capillaries are not tightly linked which may provide a route for small amounts of a hydrophilic drug to enter the brain. It is generally accepted that the ability of the molecule to pass the choroidal cells or capillary endothelial cells is a function of the lipid–water partition ratio for the molecule as described for other biological membranes. If the chemical is lipid insoluble, it will not appreciably cross the cell membrane unless it is among a group of compounds which are actively transported into the CNS, such as certain sugars and amino acids.[5]

Although it is extremely difficult for a relatively hydrophilic quaternary pyridinium salt to penetrate biological barriers, such as the blood–brain barrier, in certain instances it is extremely important to efficiently deliver through these barriers quaternary ammonium and/or pyridinium salts. For example, overcoming serious poisoning with anticholinesterase agents, such as organophosphates, is a great and increasing problem.[6,7] While pyridinecarbaldoxime type quaternary salts are the best of the blocked acetylcholinesterase (AChE) reactivating agents, among which *N*-methylpyridinium-2-carbaldoxime salt (2-PAM) is the drug of choice, the quaternary pyridinium structure of 2-PAM is one of the most disadvantageous in terms of delivery across biological membranes. The order of magnitude for the protection against an organophosphate poison conferred by 2-PAM in vivo was only a small fraction of that expected from in vitro studies of reactivation of AChE.[8] This observation has been attributed largely to the physiological distribution of 2-PAM based on its polarity. This aspect is especially important since most organophosphate poisons are very lipid-soluble and readily penetrate the CNS. Consequently, these agents efficiently deactivate the CNS acetylcholinesterase. Although 2-PAM is useful in overcoming the effects of anticholinesterase agents, its utility suffers in terms of (1) its unfavorable distribution (almost exclusively in the plasma), (2) its poor retention in the body (fast elimination, short biological half-life), (3)

† Dedicated to the memory of Professor Edward E. Smissman.