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The Pharmacology of Physostigmine

David J. Triggle,* John M. Mitchell,** and Ron Filler

*Forest Laboratories, New York, NY, *The Graduate School, State University of New York, Buffalo, NY, and **P. O. Box 5780, Somerset, NJ*

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INTRODUCTION

Physostigmine, as the salicylate salt, is being developed as a drug for the treatment of Alzheimer's disease. The memory and learning enhancement capabilities as well as other pharmacological effects of physostigmine will be thoroughly reviewed in this paper. Physostigmine (Antilirium®, Isopto®, eserine), an alkaloid from the West African perennial shrub *Physostigma venenosum*, is the oldest known acetylcholinesterase (AChE) inhibitor. Naturally occurring physostigmine was initially used clinically for ophthalmic purposes in 1877. Physostigmine was first synthesized in 1935. The general and dominant pharmacology of physostigmine is due to a short-acting inhibition of the enzymes AChE and butyrylcholinesterase (BuChE). Physostigmine exerts a stereoselective inhibition by acting as a pseudosubstrate and transferring a carbamate residue to the enzyme's active site. Spontaneous hydrolysis regenerates the native enzyme and function. This activity underlies physostigmine use in the treatment of glaucoma and atropine and organophosphate intoxication and its potential role in the amelioration of the symptoms of Alzheimer's disease.

The history of physostigmine has been reviewed by Holmstedt (120) and more recently by Somani and Dube (246). Physostigmine is extracted from the seeds of *Physostigma venenosum*. As "ordeal beans" these seeds were used in trials for witchcraft (80) and an early therapeutic use in ophthalmology was described in 1863 (12). The structure of physostigmine (1,2,3,3a,8,8a-hexahydro-1,3a,8-trimethyl-pyrrolo[2,3-b]indo-5-ol-methylcarbamate) was determined by Stedman and Barger in 1925 (251) and its effect in prolonging acetylcholine action, subsequently revealed as mediated through the inhibition of AChE (252), was discovered by Loewi and Navratil a year later (158). Physostigmine is a lipid soluble tertiary amine with a pK_a value of 7.9 and is approximately 75% ionized at the pH of blood and brain (247).

Address correspondence and reprint requests to: Dr. R. Filler, Forest Laboratories, Inc., 909 Third Avenue, New York, NY 10022-4731, USA. Fax: +1 (212) 750-9152

AChE is a hydrolytic enzyme of the serine class that is of major significance to the hydrolysis of acetylcholine in the cholinergic synapses of the somatic system, the autonomic nervous system and the central nervous system. The pharmacology of physostigmine is dominated by its interactions with this enzyme. Naturally occurring physostigmine is levorotatory (–) and the inhibition of AChE is enantioselective, with the (–) enantiomer being some 1000 times more potent than the (+) enantiomer in studies using acetylcholinesterase from human tissues (17,43; Table 1). The major metabolic products of physostigmine, including eseroline, rubreserine (Fig. 1) and the condensation products eserine blue and eserine brown are significantly less active inhibitors of AChE than physostigmine itself (17,43,116).

Physostigmine was used therapeutically in the treatment of glaucoma in 1877 (145) and its first use as an antidote in the treatment of atropine toxicity was some 125 years ago (190). The current clinical roles of physostigmine include treatment of glaucoma (172,258), myasthenia gravis (276), the relief of central cholinergic intoxication from atropine, scopolamine, and belladonna alkaloids (65,212,238,277) and from the intoxication induced by overdoses of tricyclic antidepressants (48,182,188,236), antihistamines (149), antipsychotics (27,279), and benzodiazepines (133,215). These actions all derive from the ability of physostigmine to increase acetylcholine levels by inhibition of AChE and BuChE. These increased levels facilitate neuromuscular transmission in skeletal muscle, decrease pupillary size, and increase aqueous humor outflow in the eye and block the anticholinergic effects of atropine and the anticholinergic effects of high concentrations of antihistamines, antidepressants, and antipsychotics.

Physostigmine has also gained prominence as a potential prophylactic agent against organophosphate intoxication by competition for the active sites of AChE (reviewed in 246). An early observation by Koster (144) showed that a non-lethal dose of physostigmine protected cats against the effects of several lethal doses of the organophosphate diisopropyl fluorophosphate. Many subsequent studies (59) have confirmed this observation and have demonstrated the efficacy of physostigmine prophylaxis against a variety of organophosphates, including Soman (106,117,151,175). This use would be in conjunction with post-exposure anticholinergic and oxime therapy.

Pyridostigmine bromide, a quaternary charged carbamate cholinesterase inhibitor, has been advanced by the military as an orally active protective drug against organophosphate exposure under battlefield conditions (76,138). Pyridostigmine does not

TABLE 1. Inhibitory activities of physostigmine analogs against cholinesterases[1]

	Human AChE			Human BuChE	
	cortex	caudate	erythrocyte	cortex	plasma
(–)Physostigmine	31	35	28	129	15
(±)Physostigmine	72	74	70	265	35
(+)Physostigmine	22,000	26,000	25,000	26,000	4000
Eseroline	> 10,000	> 10,000	> 10,000	> 10,000	> 10,000

[1] $IC_{50} \times 10^{-9}$ M. Data from refs. 17 and 43.
Abbreviations: ChE, cholinesterase; BuChE, butyrylcholinesterase.

Fig. 1. Structures of physostigmine, eseroline, and rubreserine.

cross the blood-brain barrier, however, and hence does not protect against performance deficits produced by central cholinesterase intoxication. Physostigmine, because of its ability to cross the blood-brain barrier, has been demonstrated experimentally to be more effective than pyridostigmine against Soman poisoning (178).

Physostigmine has also generated considerable attention in both experimental and clinical protocols as a memory- and cognition-enhancing agent (67,221,273). The role of the central cholinergic nervous system in memory processes was summarized by Deutsch in 1971 and in 1983 (73,74). The role of physostigmine in the memory process has assumed increasing importance with many observations indicating that degeneration of central cholinergic neurons in the basal forebrain is associated with geriatric memory dysfunction and with Alzheimer's disease (reviewed in 24,64,173,184). The cholinesterases are the principal pharmacological targets of physostigmine and the pharmacological effects of administered physostigmine are almost entirely due to its actions on these enzymes.

CHOLINESTERASES

Properties and Distribution

Acetylcholine is hydrolyzed by AChE EC 3.1.1.7 or true cholinesterase (291). The AChEs are associated primarily with nerve and muscle, typically at synaptic contacts. BuChE EC 3.1.1.8, or pseudocholinesterase, is synthesized in liver and also hydrolyzes higher choline esters, including propenyl- and butyrylcholine. BuChE has a widespread distribution in serum, glia, and neurons, although its physiological function remains to be defined (53,163,165,256,257).

Genes and Proteins

Both AChE and BuChE are polymorphic and exist as homomeric and heteromeric molecular forms characterized by their subunit associations and hydrodynamic properties (reviewed in 256,257). The heteromeric molecular forms contain catalytic subunits linked to lipid or a triple helical collagen tail and are frequently referred to as the asymmetric or A forms of AChE. The homomeric hydrophilic globular forms of AChE, G1, G2, and G4, contain one, two, or four identical subunits, respectively. The G4 form is secreted by neurons and secretory cells. An amphophilic glycophospholipid-linked form is a dimer of two subunits (G2) with a glycophospholipid link to the cell membrane. Less polymorphism is seen with BuChE with only hydrophilic and asymmetric forms having been identified. The distribution of the various forms of the cholinesterases is tissue dependent.

AChE and BuChE share > 50% amino acid residue identity and appear to have identical or very similar three-dimensional structures (108). Both AChE and BuChE are encoded by unique single genes: human AChE is localized to 7q22 on chromosome 7 and human BuChE to 3q26 on chromosome 3 (5,94). Despite their similarity, AChE and BuChE display a number of important biochemical differences, including substrate specificity, substrate inhibition, and antagonist interactions. AChE is almost completely specific for acetylcholine and is inhibited by high acetylcholine concentrations; in contrast, BuChE hydrolyses a wide variety of substrates and is activated by substrates (156,157). Because of the broader ligand sensitivity of BuChE it has been described as a drug scavenger (240).

In human brain, methanesulfonyl fluoride was selectively inhibitory for AChE in comparison to BuChE (second-order rate constants 110 and 6.9/M/min, respectively) and physostigmine was modestly selective (second-order rate constants of 6.3×10^5 and 1.8×10^{-5}/M/min for AChE and BuChE, respectively) (201).

The role of polymorphism of both AChE and BuChE with respect to enzyme function is not defined, but both the total AChE activity and the ratio of the G4 to the G1 form of AChE vary widely in human brain, whereas BuChE shows significantly less regional and molecular variation (15–17). Little is known of the relative sensitivity of the several molecular forms of the cholinesterases to inhibitors. Ogane et al. (196) found that physostigmine was not differentially active against the G4 or G1 forms of rat brain AChE; however, heptylphysostigmine and neostigmine showed greater inhibitory activity toward the G1 form.

Mechanisms of Action

The structure of AChE reveals an active site containing a catalytic triad — glutamate (E_{327}), histidine (H_{440}), and serine (S_{200}) — located at the base of a narrow gorge some 20 Å in depth. This general arrangement of amino acids is representative of the serine hydrolase family of enzymes. The gorge in AChE is lined with 14 aromatic amino acids, consisting of phenylalanine, tyrosine, or tryptophan, and the base of the gorge contains several anionic residues that collectively are responsible for both interaction with the positively charged trimethylammonium group of acetylcholine and the

Fig. 2. The interactions of acetylcholine and physostigmine at acetylcholinesterase.

acceleration of the binding of cationic ligands (reviewed in 257). BuChE contains six fewer aromatic amino acids in the gorge.

From these structural studies emerges a view of the molecular basis of ligand specificity at the active center (257). In particular, the major substrate distinction between AChE and BuChE is likely determined by the relative roles of two phenylalanines (F_{288} and F_{290}) that provide a rigid acyl binding pocket in AChE and their replacement in BuChE by leucine and valine to provide a less structurally constrained pocket. Additionally, AChE contains a peripheral anionic site that is responsible for the allosteric inhibition by cationic ligand interactions at the catalytic site. This peripheral anionic site, proposed by Changeux in 1966 (52), binds agents such as propidium at residues around the rim of the gorge (6,28,257). This peripheral anionic site may play a role in the catalytic process by mediating substrate inhibition.

Acetylcholine is hydrolyzed via an intermediate and highly labile acetylated enzyme (Fig. 2; Equation (1)):

$$\text{AChE–OH} + \text{CH}_3\text{COOR} \overset{k_1}{\leftrightarrow} \text{AChE OHCH...COOR} \overset{k_2}{\rightarrow} \text{AChE–OOCCH}_3$$

$$+ \text{ROH} \overset{k_3}{\rightarrow} \text{AChE–OH} + \text{CH}_3\text{COO}^- + \text{H}^+ \qquad (1)$$

In this scheme (145):

$$k_{cat} = \frac{k_2 k_3}{k_2 + k_3}; \; k_m = \frac{k_{-1} + k_2}{k_1} \frac{k_3}{k_2 + k_3}; \; \frac{k_{cat}}{k_m} = \frac{k_1 k_2}{k_{-1} + k_2}. \qquad (2)$$

This mechanism resembles that of other serine esterases and involves a nucleophilic attack by a specifically polarized serine residue to yield a tetrahedral intermedi-

Fig. 3. Structures of pyridostigmine and other cholinesterase inhibitors. DFP, diisopropylfluorophosphonate.

ate that collapses to yield the acetylated enzyme. In turn, this acetylated enzyme is rapidly hydrolyzed to generate free acetate (164,165,211,256,257,283). AChE has an extremely high turnover rate, $k_{cat}/k_m = 10^8/M/sec$, which is approximately 10^6 ACh molecules per molecule of enzyme per minute with a 10^4 rate enhancement over water-catalyzed ester hydrolysis. For acetylcholine k_{cat} approaches 10^4 sec and $k_m = 5 \times 10^{-5}$ M; accordingly, k_{cat}/k_m approaches the diffusion-controlled limit.

For efficient substrates, such as acetylcholine, the hydrolysis rate is determined by the diffusion of substrate to the active site; for less efficient substrates the rate may depend either on the acylation step or the isomerization steps of the enzyme leading to acylation. For carbamylating (and phosphorylating) agents, including physostigmine, deacylation (k_3) becomes the rate-limiting step. Inhibition of this enzyme may lead to a rapid increase in local acetylcholine concentrations. This mechanism underlies the pharmacologic, therapeutic and toxicologic properties of drugs that inhibit AChE, including physostigmine, neostigmine, pyridostigmine and DFP (Fig. 3).

PHYSOSTIGMINE

Absorption, Distribution, Metabolism, and Excretion

Despite the long history of study of the pharmacologic properties of physostigmine, relatively few pharmacokinetic studies have been carried out. Pharmacokinetic studies with physostigmine have been conducted in several animal species including rat (35,239,241,242,244,247,265), guinea pig (160), and dog (95). Physostigmine and physostigmine metabolite (Fig. 1) concentrations can be measured by HPLC (42,141,148,176,177,230,280) and by radioimmune assay (176,177). From these studies, it has been determined that physostigmine has a high clearance, is eliminated almost entirely via metabolic pathways, and has a high hepatic extraction ratio. Hepatic blood flow is a principal determinant of physostigmine clearance (265).

Autoradiography studies in rats and positron emission tomography (PET) studies in primates have provided *in vivo* visualization of cholinesterase by the use of [^{11}C]labeled physostigmine (255). The studies were uncomplicated by metabolism because the [^{11}C] was located on the carbamate residue of physostigmine. Clear regional differences in physostigmine disposition in the brain were found in both rats and primates and the cerebral uptake of the [^{11}C] species was significantly reduced by competition with unlabeled material. In rat brain, physostigmine disposition, following intravenous administration, was highest in striatum, moderate in the cortex and hippocampus, and least in the cerebellum. Autoradiographic studies showed an exact correlation with the distribution of AChE staining. The mean cerebral uptake of physostigmine expressed as a percentage of the injected dose per gram of tissue (% IDPG) was 0.4% at 5 and 30 min and 0.25% at 60 min. The ratios of striatal: cerebellar radioactivities were 1.7, 2.1, and 2.7 at 5, 30, and 60 min, respectively. In primates, after a bolus intravenous injection of [^{11}C]physostigmine, radioactivity peaked in the blood during the first minute and then declined rapidly ($t_{1/2}$ = 0.58 min). Sixty minutes after injection, blood radioactivity was 8.5% of the injected dose. In the brain, using PET, it was determined that the peak of radioactivity was achieved some 2 to 3 min after injection and reached 33% IDPG in the putamen, 28% in the caudate nucleus, 27% in the cerebellum, and 24% in the whole cerebral cortex. Twenty minutes following injection, the striatum showed the highest level of radioactivity, followed by the cortex and cerebellum.

The metabolism and disposition of physostigmine in the rat have been measured following intramuscular, intravenous, and oral administration (239–242,265). The dominant route of metabolism is through the liver. Unni and Somani (265) have reported that about 90% of physostigmine is metabolized by the liver within 2 min of administration. Metabolism is the major route of elimination of physostigmine (239) rather than urinary or biliary excretion (35,239). The liver plays a particularly important role in the metabolism of physostigmine (265). The extraction ratio (ER) and intrinsic clearance (CL_{int}) from liver and skeletal muscle following intravenous administration are summarized in Table 2. The ER is defined as $(C_A - C_V/C_A)$, where C_A is the concentration of drug in arterial blood and C_V is the concentration in venous blood.

Following intramuscular administration of [^{3}H]physostigmine (650 μg/kg), the accumulation of radioactivity was highest in the liver and kidney, and less than 1% of the initial amount remained in these tissues after 7 d (241). The plasma physostigmine concentration showed a peak level of 582 ± 35 ng/ml at 5 min. The metabolites found were eseroline, M_1, and M_2 (M_1 and M_2 are of undefined structure). M_1 was the major metabolite and eseroline and M_2 were of minor significance. Metabolism was rapid, the half-lives of physostigmine in plasma and brain being 16 and 17 min, respectively, and the brain to plasma ratio, 1.61, peaked at 22 min. Pharmacokinetic constants for intramuscular administration of physostigmine are listed in Table 3.

Following intravenous administration (100 μg/kg) of physostigmine in the rat, the major metabolite M_1 reached a peak at 30 min: eseroline and M_2 were minor metabolites (240–242). A summary of the pharmacokinetic parameters in the rat following intravenous administration is presented in Table 4. After 60 min only 1.5% of the original radioactivity was present in the plasma, 85.1% was M_1, and 5.4% was parent drug. Tissue distribution of physostigmine showed that the highest concentrations expressed as radioactivity/gram of tissue were reached in kidney and liver. The percentage of the administered radioactivity present in the various tissues was highest in muscle,

TABLE 2. Extraction ratios and clearances of physostigmine following 100 μg/kg intravenous administration in rats

TABLE 3. Pharmacokinetic constants of physostigmine in rats following intramuscular administration[1]

followed by liver and plasma. Physostigmine concentrations in the plasma reached a peak of 83.3 ng/ml at 2 min and declined to 3.3 ng/ml by 60 min. Physostigmine concentration in the brain was higher than that achieved in plasma and reached a peak of 128.5 ng/kg at 3 min and declined to 3.3 ng/kg at 60 min. The time course of physostigmine decline in the plasma was biphasic. The half-lives of plasma elimination were 1.3 and 15 min (α- and β-half-lives). The apparent volume of distribution, V_d, in the rat was 1.35 l/kg and is indicative of tissue sequestration and is similar for other species including dog and human (95,113).

The half-lives of physostigmine in the liver and skeletal muscle of the rat were 24 and 20 min, respectively, following intravenous administration, and the elimination rate constants were 0.0288 and 0.0351/min (265). There is a high clearance of physostigmine in the rat, 62 ml/kg, which is higher than the clearance in dogs or humans and probably relates to a higher rate of metabolism of physostigmine in the rat. The concentration of physostigmine in the brain was higher than that in the plasma at all measured time points.

Physostigmine disposition has also been studied in the rat following oral administration of 650 μg/kg (244). The distribution of radioactivity was maximum at 15 min in liver and kidney and then declined: a similar temporal pattern was found in other tissues including brain, muscle, heart, spleen, and lung, although the maximum extent of radioactivity accumulation was 10- to 40-fold less in these tissues. Radioactivity on a per-organ basis was maximum in the liver following oral administration, in contrast to the pattern seen after intravenous or intramuscular administration. This is consistent with a complete absorption of physostigmine after oral administration followed by first-pass metabolism. Physostigmine concentrations achieved in other tissues were low. A peak plasma concentration of 2.89 ng/ml was achieved at 15 min and had declined to 0.71 ng/ml at 90 min. Physostigmine concentration in the brain peaked at 22 min at a concentration of 2.85 ng/mg and then declined to 0.33 ng/mg by 60 min. The pharmacokinetic parameters for physostigmine following oral administration (650 μg/kg) in the rat are presented in Table 5 (244). The bioavailability (F) value, the fraction of the dose that reaches the systemic circulation, is 0.02 and the high extraction ratio, 0.98, is consistent with a first-pass effect (244). The hepatic clearance was calculated to be 31.28 ml/min/kg.

TABLE 4. Pharmacokinetic constants of physostigmine in rats following intravenous administration[1]

The clearance of physostigmine by rat liver and skeletal muscle has been compared following intravenous and intramuscular administration (265). The half-lives in liver after intravenous and intramuscular administration of 100 μg/kg and 650 μg/kg, respectively, were very similar at 24 and 26 min, indicating that physostigmine does not exhibit dose-dependent kinetics.

The pharmacokinetics of physostigmine have been measured in the guinea pig following intramuscular administration in the dose range of 4 to 146 μg/kg (160). Physostigmine is absorbed, distributed, and eliminated rapidly with linear pharmacokinetics within the dose range employed. The pharmacokinetic values are summarized in Table 6. At all dose levels, physostigmine plasma levels peaked at approximately 30 min (T_{max}). The apparent volumes of distribution, approximately constant a 2 l/kg are in accord with those in rat, dog, and human (95,113,281), and are consistent with tissue sequestration of physostigmine.

The disposition of physostigmine has been studied in beagle dogs following intravenous bolus administration at 100 μg/kg (95). The distribution half-life was short,

TABLE 5. Pharmacokinetic constants of physostigmine in rats following oral administration[1]

TABLE 6. Pharmacokinetic constants of physostigmine in guinea pigs following intramuscular administration

2.2 min, consistent with other species, and equilibration of the drug in organs was rapid. The metabolites were eseroline, M_1 and M_2; M_1 is the major metabolite. The distribution of physostigmine in brain regions revealed a fairly uniform distribution. However, the hippocampus and temporal cortex showed higher concentrations than other regions, 28.4 and 21.9 ng/g, respectively, at 70 min following physostigmine administration. In the plasma, at 45 min, 18% of the radioactivity was due to physostigmine and 52% was due to metabolite M_1. This contrasted with the brain, where at 70 min only 1.9 to 3.4% of radioactivity was due to metabolite M_1. The plasma concentration of physostigmine peaked at 2 min (T_{max}) and subsequently decreased, while the concentration of M_1 peaked at 45 min. Physostigmine clearance from the plasma was a biexponential process. The pharmacokinetic parameters are shown in Table 7.

A comparison of pharmacokinetic parameters in three species (rat, dog, and human) is presented in Table 8 (95). Several species differences are apparent. The mean elimination half-life, $t_{1/2\beta}$, is greater in dog (30.7 min) than in rat (15.0 min), or humans (21.7 min). Similarly, the apparent volume of distribution is greater in the dog and rat than in humans, although in all three species V_d is greater than total body volume

TABLE 7. Pharmacokinetic constants of physostigmine in dogs following intravenous administration

TABLE 8. Comparison of some pharmacokinetic constants of physostigmine in three species following intravenous administration

	Species		
	rat	dog	human

consistent with tissue sequestration of physostigmine. Additionally, clearance is greater in the rat. Since physostigmine is eliminated almost entirely through metabolic pathways, this difference may be due to a higher metabolic rate in the rat. Regardless of species, clearance and metabolism through the liver is the most important dispositional pathway for physostigmine.

Binding to Plasma Proteins

Physostigmine is bound to serum proteins although estimates of binding affinity and capacity differ markedly (243,264,282). In rat plasma, physostigmine binding decreased slightly from 49% to 41% with increasing physostigmine concentration over a 50-fold range from 0.16 to 8.1 nmol/ml, whereas binding increased in human plasma from 29% to 43% over the same concentration range. Scatchard analysis revealed a positive slope for human but a negative slope for rat plasma with binding constants, K_1 and K_2, of approximately 5×10^5 and 5×10^4 l/mol, respectively; these slopes may suggest the presence of positively and negatively cooperative binding processes. In sera, binding ranges from 29% to 49% were observed, but the binding of physostigmine to crystalline albumin, gamma-globulin and α_1 acid glycoprotein was much lower, suggesting that the majority of physostigmine binding is to high- or low-density lipoproteins. This view is supported by the observation that quinidine, which binds to all lipoproteins (191), displaces to a considerable extent physostigmine bound to human serum (243).

The binding of physostigmine to human serum albumin has been characterized by Whelpton and Hurst (282). Over the concentration range 3.3 nM to 2.7 μM, a single class of binding sites was determined with an affinity constant of 8×10^7 l/mol and a density of approximately 3 nM. This affinity is significantly higher than that measured by Unni and Somani (264) and it may represent binding to trace cholinesterase in the human plasma studied. Protein-bound physostigmine may contribute to drug interactions (243,264,265). Human plasma treated with an anticoagulant (citrate phosphate dextrose adenine-1 solution) bound approximately 50% less physostigmine than untreated plasma (265). The binding of physostigmine to plasma proteins from normal human volunteers was measured in the presence of a number of commonly prescribed drugs (243). The binding of physostigmine, 0.2 μg/ml, decreased from 45.5% alone to 5.3% in the presence of quinidine (3 to 15 μg/ml), to 28.4% in the presence of furosemide (1 to 100 μg/ml), to 37.9% in the presence of acetaminophen (10 to 160 μg/ml), to 43.7% in the presence of theophylline (6.67 to 80 μg/ml), and to 34.9% in the presence of verapamil (0.1 to 2 μg/ml). The clinical significance of these observations is not clear; however, increases in the free fraction of plasma physostigmine could contribute to enhanced toxicity of physostigmine.

CONCENTRATION – RESPONSE RELATIONSHIPS

Several studies have determined the relationship between physostigmine concentration and cholinesterase activity in blood and tissues (75,95,106,107,110,111,169,242,245). In human brain postmortem tissue, physostigmine had IC_{50} values of 14 to 15 nM in

frontal cortex and hippocampus and was equally potent in other tissues, including erythrocytes and cortex from neurosurgical patients (204,260). Data from the comparative study by Somani and Dube (245) in rat tissues are summarized in Table 9. Dose-dependent relationships between physostigmine concentration and cholinesterase inhibition were found in erythrocytes, brain, diaphragm, heart and skeletal muscle. Thus, physostigmine produces a dose-dependent inhibition of erythrocyte cholinesterase in the concentration range 25 to 200 μg/kg; at higher doses no further inhibition was observed.

Similarly, maximum cholinesterase inhibition by physostigmine occurred in the brain at about 150 μg/kg. Measurements of brain concentrations of physostigmine revealed a linear relationship between dose and tissue accumulation (Fig. 4).

Somani and Khalique (242) have demonstrated linear relationships after intravenous administration between plasma physostigmine concentrations and BuChE and AChE activities in rat plasma and brain, respectively (Fig. 5).

The effect of route of administration (i.m., i.v., and i.c.v.) of physostigmine on brain, plasma, and erythrocyte cholinesterase levels has been determined in the rat by Hallack and Giacobini (106,107), with doses of 500 and 100 μg/kg, and 4 μg/kg for i.m., i.v., and i.c.v. routes, respectively. Following intramuscular administration at 500 μg/kg, which produced 14% mortality, whole brain cholinesterase activity was maximally inhibited (76%) at 5 min, and after 40 min the inhibition was reduced to 50%. The regional decrease (%) in cholinesterase levels was cortex > cerebellum > hippocampus > medulla > striatum, which parallels approximately the physostigmine levels of cortex hippocampus > striatum > cerebellum > medulla. The time courses of cholinesterase activity and physostigmine levels are shown in Fig. 6*A,B*. Following intravenous administration, a similar inverse relationship was found between brain physostigmine levels and cholinesterase activity, and the regional distribution of cholinesterase inhibition was very similar to that observed following intra-

TABLE 9. Dose-response relationship for physostigmine inhibition of cholinesterase activity in rat tissue[1]

Fig. 4. Relationship between physostigmine concentration (●) and % ChE inhibition (○) in brain of rats administered physostigmine 50 to 500 μg/kg i.m. Abbreviations: ChE, cholinesterase. Reproduced with permission from reference 244, Somani SM. Pharmacokinetics and pharmacodynamics of physostigmine in the rat after oral administration. *Biopharm Drug Dispos* 1989;10:187–203.

Fig. 5. Relationships between physostigmine concentration (ng/ml) and (*A*) BuChE activity in rat plasma; (*B*) BuChE activity in rat brain; (*C*) physostigmine concentration in plasma and ChE inhibition in brain. Abbreviations: BuChE, butyrylcholinesterase; ChE, cholinesterase. Reproduced with permission from reference 244, Somani SM. Pharmacokinetics and pharmacodynamics of physostigmine in the rat after oral administration. *Biopharm Drug Dispos* 1989;10:187–203.

muscular administration. Intracerebroventricular administration yielded a similar regional sensitivity. However, the physostigmine distribution was very non-uniform, with the highest concentrations being found in the hippocampus and striatum (Table 10).

Fig. 6. *A*. Time course of ChE activity in rat brain after administration of physostigmine 500 μg/kg i.m. Inset: Relationship between % ChE activity to brain and in plasma or RBC. Abbreviations: ChE, cholinesterase; RBC, red blood cells. *B*. Time course of the concentration of physostigmine in rat brain after administration of physostigmine (500 mg/kg i.m.). Abbreviations: ST, striatum; T, septum; MO, medulla; HC, hippocampus; CX, cortex; CR, cerebellum. Reproduced with permission from reference 107, Hallak M, Giacobini E. A comparison of the effects of two inhibitors on brain cholinesterase. *Neuropharmacology* 1987;26:521–530.

TABLE 10. Cholinesterase activities and physostigmine concentrations in rat brain areas after administration of physostigmine[1]

Effect on ACh Concentration

Many studies have demonstrated that physostigmine elevates cholinesterase concentrations both peripherally and centrally. Hallack and Giacobini (106,107), have shown that cholinesterase concentration peaks in the rat brain some 20 min after the peak of cholinesterase inhibition, which is a level approximately 2- to 5-fold higher than control. The actual change in cholinesterase concentration is region-dependent: the striatum, which shows the largest absolute decrease in cholinesterase activity, shows the largest increase in acetylcholine level (Fig. 7*A*,*B*). Infusion of physostig-

Fig. 7. *A.* Relationship between ChE activity and physostigmine dosage in rat brain regions. Abbreviations: ST, striatum; SP, septum; MO, medulla, HC, hippocampus; CX, cortex; CR, cerebellum. *B.* Time course of acetylcholine concentration in rat brain areas after physostigmine administration (650 μg/kg i.m.). Reproduced with permission from reference 106, Hallak M, Giacobini E. Relation of brain regional physostigmine concentration to cholinesterase activity and acetylcholine and choline levels in rat. *Neurochem Res* 1986;11:1037–1048.

mine into rat brain cortex at two dose levels, 30 and 300 μg/kg, produced a dose-dependent increase in cholinesterase with a peak between 30 and 60 min and a peak inhibition of cholinesterase at 30 min (66).

Physostigmine (0.5 mg/kg, i.p.) decreases acetylcholine synthesis in rat brain presumably as a consequence of increased acetylcholine concentrations (32). At concen-

trations higher than those needed to block AChE, physostigmine also increases acetylcholine release by blocking K^+ currents (114). In cats, physostigmine (100 μg/kg, i.v. or s.c.) elevated brain acetylcholine content, particularly in the neocortex, caudate nucleus and hippocampus, by 10% to 30% and in rat whole brain and cerebral cortex by 25 to 40% (22). Regionally selective increases in brain acetylcholine were also reported in the rat brain by Hallak and Giacobini (106) and in the guinea pig brain, where the largest increases occurred in the olfactory lobes, the parietal cortex, and the caudate nucleus. Choline acetylase (ChAT) activities increased in all regions, coincident with a decline in cholinesterase activity (171). Presumably, the increased ChAT levels are a reflection of the increased acetylcholine concentration. Similarly, physostigmine (7.7 μM) approximately doubles the acetylcholine content of the guinea pig ileal-myenteric plexus preparation (125). A correlate to these studies is the increased acetylcholine release seen in brain slices from Alzheimer patients after exposure to high concentrations (10^{-5} to 10^{-4} M) of physostigmine (192).

Pharmacokinetic and Pharmacodynamic Model

A physiological pharmacokinetic and pharmacodynamic model for physostigmine in the rat has been developed (247). This model, based on organ perfusion and volume data, predicts organ drug concentrations and dynamics as a function of time. The underlying assumptions were those of a multicompartment model with physostigmine clearance by liver and muscle only, with two major pools in the plasma and brain and a separate pool representing adipose, intestine, kidney, and eye tissues. For the development of the pharmacokinetic model it was further assumed that each tissue acts as a well-stirred compartment, that physostigmine distribution is flow limited, that the tissue: plasma partition coefficients are concentration independent, and that all processes are first order. Using experimentally available values for plasma flow through the pools, tissue volumes, partition coefficients, and clearance data, the model yielded a satisfactory correlation between predicted and observed plasma concentrations of physostigmine and inhibition of brain cholinesterase activity (Fig. 8).

A similar agreement was found between calculated and observed changes in brain cholinesterase activity and brain physostigmine concentrations. A summary of the physiological and experimental parameters used in the construction of the model for physostigmine transport is provided in Table 11.

Nonclassical Roles for Cholinesterases

In addition to its role in hydrolyzing acetylcholine, AChE may exhibit a number of nonclassical roles (reviewed in 9). AChE is secreted from several brain regions, including hippocampus, cerebellum, cortex, and substantial nigra in both cholinergic and noncholinergic pathways (8,9,71,105,237). Neuronally secreted AChE is the source of the enzyme found in cerebrospinal fluid. There are suggestions that secreted AChE has a number of noncholinergic functions, including activation of ATP-sensitive K^+ channels in Purkinje neurons and enhancement of excitatory amino acid action

Fig. 8. Simultaneous plasma physostigmine concentration-time (○) and the effect measured as the change of cholinesterase activity in the brain (●) after intravenous administration in the rat ($n = 4$). The lines (solid line for the plasma and broken line for the effect) represent the theoretic lines obtained by the curve-fitting procedure of the parametric PK-PD model. Reproduced with permission from reference 247, Somani SM, Gupta SK, Khalique A, Unni LK. Physiological pharmacokinetic and pharmacodynamic model of physostigmine in the rat. *Drug Metab Dispos* 1991;19:655–660..

(10). Additionally, AChE may also have tryptic-like activity, which suggests that it has a role as a secreted protease with function in neuronal development (8,9,237). While these roles remain speculative, they may be relevant to degenerative disorders such as Alzheimer's disease, where loss of AChE occurs in both cholinergic and non-cholinergic areas.

TABLE 11. Parameters in rat used in mass balance equations of PK-PD model to describe the transport of physostigmine in the rat

CHANGES IN CHOLINESTERASES DURING DISEASE

Changes in cholinesterase during neuronal degeneration have received significant attention. A selective loss of the G4 form occurs in post-mortem brain from Alzheimer's patients (15). Additionally, changes in enzyme distribution and substrate properties have been reported (71,174,175,227). In brains from Alzheimer patients, there is a shift in both AChE and BuChE activities away from the neuronal pathways to the neuritic plaques and neurofibrillary tangles. These enzymes were potently inhibited *in vitro* by physostigmine (174). Additionally, the pH optimum of the Alzheimer brain enzymes in the acetylcholine staining procedure was shifted from the normal pH of 8.0 to lower pH values of 6.8 to 7.0. The sensitivity to the inhibitor BW284C51 was approximately 100-fold less (10^{-5} to 10^{-4} M for complete inhibition) than for the control enzyme (71) and enzymes were less sensitive to substrate inhibition, by a factor of 2- to 4-fold, for both acetylcholine and butyrylcholine (227). These observations suggest that the clinical effects of anticholinesterases in Alzheimer's disease may be based on mechanisms involving modified kinetic parameters compared to those that apply to the normal brain (174). This apparent change in AChE action may offer a basis for some selectivity of cholinesterase inhibitors in Alzheimer's disease.

The change in distribution of enzyme activities may relate to cholinergic function in disease states (16,19,123,276). In particular, the occurrence of cholinesterase in cerebrospinal fluid (CSF) is of importance in providing a marker of brain enzyme levels in degenerative disorders and serves as a measure of the extent of both brain enzyme levels in degenerative disorders as well as the extent of brain cholinesterase inhibition by drugs. AChE activity in CSF is proposed to be of neuronal origin (20,55,95–97) and BuChE presumably originates from both. brain and blood. Although the relative enzyme levels of AChE are significantly lower in CSF than in plasma, the specific activities are actually higher (Table 12). Both G2 and G4 forms have been reported in human CSF; however, whether changes in AChE levels in CSF are a useful marker for brain changes remains to be determined (82).

INTERACTION OF PHYSOSTIGMINE WITH CHOLINESTERASES

Physostigmine interacts with cholinesterase enzymes as a competitor or pseudo-substrate. It is hydrolyzed through the same mechanism as that for the physiologic transmitter acetylcholine, but the intermediate drug-enzyme complex is more stable. This prevents endogenous acetylcholine from reacting with the enzyme and thus preserves or enhances the local concentration of AChE.

The Kinetics of Drug–Enzyme Interactions

The hydrolytic mechanism of the cholinesterases employed against the physiological substrate, acetylcholine, is shared by physostigmine and other carbamates. Physostigmine interacts at both anionic and esteratic sites of the enzyme catalytic triad to yield the intermediate carbamylated enzyme (Fig. 2); this intermediate is, however, hydrolyzed comparatively slowly and the hydrolysis of acetylcholine is accordingly impaired, because the esteratic site of the enzyme is occupied by the N-methylcarbamyl residue (71,98,102,103,110,246,258). Kinetic constants have been determined for physostigmine (IX) interaction with rabbit brain acetylcholinesterase (EH) according to the following three step process:

$$EH + IX \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} EH.IX \xrightarrow{k_2} EI + HX \xrightarrow{k_3} EH + IOH$$

with k_{-1}/k_1 or $[K_D] = 7.1 \times 10^{-6}$ M, $k_2 = 0.59$/sec, the inhibitory constant, $k_i = k_2/K_D = 8.3 \times 10^{-4}$ and $k_3 < 0.59$/sec (102).

The rates of carbamylation of cholinesterase *in vivo* by physostigmine will be dependent upon the concentrations of physostigmine achieved at the tissue: this is determined by blood flow, partition coefficient, plasma protein binding, and pH (246). Somani and Khalique reported the rates of decarbamylation of AChE in rat brain and muscle and of BuChE in plasma to be 0.027, 0.083, and 0.011 min, respectively

TABLE 12. Acetylcholinesterase activities in canine cerebral spinal fluid and plasma

(241,242). The differences in decarbamylation rates between tissues reflect microenvironmental factors, including tissue pH. Different molecular features of the enzyme determine the interaction of physostigmine with AChE and BuChE. In a series of N-substituted and carbamoyl derivatives of physostigmine, activity against AChE increased with increasingly hydrophobic N-substituents. In contrast, activity against BuChE increased with increasingly hydrophobic N-substituents and carbamoyl substituents (18,162).

Inhibition of rat brain acetylcholinesterase by physostigmine *in vivo* was shown to be region selective (199). At low concentrations of physostigmine (50 nM) the percent of AChE activity decreased by 5.7%, 4.6%, 10.1%, 8.0%, 8.4%, and 25% of the control values in the whole brain, cerebellum, pons, frontal cortex, basal ganglia, and medulla oblongata, respectively. These differences may reflect the different proportions of white and gray matter in different brain regions, since white matter contains more lipid than gray material (83).

GENOMIC VARIATIONS IN CHOLINESTERASE SENSITIVITY TO DRUGS

Serum BuChE likely functions as a drug scavenger for esterase-susceptible drugs, including therapeutic agents such as the neuromuscular relaxant succinylcholine. Individuals bearing atypical BuChE will show excessively prolonged apnea following succinylcholine therapy and will be characterized by insensitivity to dibucaine (reviewed in 135). This atypical phenotype was characterized by a single mutational change of Asp-70 to Gly at the active site of BuChE (170). This particular mutation represents the variant most frequently found in patients who respond abnormally to succinylcholine. It occurs in homozygous form in 1 in 3500 Caucasians. A number of other human BuChE mutants are now known with pharmacological properties that differ from the wild-type enzyme, including decreased binding and sensitivity to carbamate inhibitors (154–157,187).

The general properties of the atypical cholinesterase are that it shows reduced affinity to positively charged drugs, but exhibits the same turnover number (154,155). Thus, the IC_{50} values for physostigmine at the normal and atypical enzymes are 1.4×10^{-8} M and 2.7×10^{-7} M, respectively. Individuals bearing atypical cholinesterase should be less sensitive to the effects of physostigmine. However, there appears to be little, if any, clinical data bearing on this point.

GENERAL MIMICRY OF ACETYLCHOLINE EFFECTS

As a substrate competitor of cholinesterase, the dominant pharmacological effects of physostigmine may be anticipated to be identical or similar to those of the physiologic cholinergic transmitter, acetylcholine. In general, this is true, but the extent of the mimicry of acetylcholine action depends upon a number of factors, including the distribution of physostigmine. Physostigmine is a tertiary amine with pKa of 7.9, which at physiologic pH exists as both the protonated form and as the lipid-soluble

free base. Structural analogs of physostigmine, including neostigmine and pyridostigmine, which are permanently positively charged, do not penetrate the CNS and are devoid of the centrally mediated actions of physostigmine, including action at central cholinergic sites. Other factors controlling the pharmacological behavior of physostigmine include the general pharmacokinetic behavior, route of administration, and, of particular importance, the extent to which a particular cholinergic pathway is active and thus susceptible to the potentiating action of AChE inhibition. Therefore, the effects of physostigmine on vascular smooth muscle are much less marked than the effects of directly acting muscarinic agonists, because vascular smooth muscle is not directly innervated by cholinergic nerves. The effects of physostigmine on blood pressure are also less than those of directly acting agonists because the net effects of physostigmine on vascular tone may represent activation of both the sympathetic and parasympathetic divisions of the autonomic nervous system. Physostigmine shares the electrophysiological consequences of acetylcholine action. This has been demonstrated for both the nicotinic and muscarinic actions of acetylcholine. At nicotinic receptors of skeletal muscle the twitch potentiations and fasciculations mediated by physostigmine are due to presynaptic actions of acetylcholine, while postsynaptic actions are revealed in the enhancement and prolongation of the end plate potential and the associated tension responses (26,283,284).

Effects on Memory

The central cholinergic nervous system has long been associated with learning and memory in both humans and animals (24,73,74,184). The effects of physostigmine have been examined experimentally in a variety of memory paradigms, passive, active, and those induced by cholinergic deficits and lesions. These effects have been reviewed (81,184).

In passive avoidance trials with rats and mice (109,118,127,222–224,250,286), physostigmine has been shown to enhance the learning process. Physostigmine is usually more effective at lower rather than higher doses. Haroutunian et al. (109) using a wide range of doses (0.03 to 0.12 mg/kg i.p.) reported that 0.03 mg/kg physostigmine was the most effective dose studied, and that higher doses were not more effective than saline (Fig. 9).

Dumery et al. (81) observed that passive avoidance learning could be acquired in young rats (7 to 20 days of age). This acquisition could be facilitated with low doses of physostigmine (0.1 to 0.2 μg) bilaterally injected into the lateral amygdaloid nucleus; higher doses were without effect and progressively impaired acquisition. Low doses of physostigmine (0.015 to 0.03 mg/kg i.p.) also enhanced both memory consolidation and memory retrieval processes in rats (224). These effects could not be attributed to any obvious peripheral cholinergic side effects of physostigmine that might have altered motor responsiveness.

Nootropic agents, including piracetam and oxiracetam, improved learning in a number of experimental situations (189). These agents facilitated active, but not passive, avoidance learning, but did not enhance the avoidance-improving properties of physostigmine (222,223,226). This suggests that there is no advantage in combining these two classes of drugs.

Fig. 9. The effects of the indicated intraperitoneal doses of: *A*, physostigmine; *B*, 4-aminopyridine; *C*, oxotremorine on 72-h retention of passive avoidance response. Reproduced with permission from reference 109, Haroutunian V, Barnes E, Davis KL. Cholinergic modulation of memory in rats. *Psychopharmacology* 1985;87:266–271.

Deficits in passive avoidance learning can be produced by both anticholinergic drugs and surgical lesions. Systemically administered pirenzepine or scopolamine induces a passive avoidance learning deficit in rats that can be antagonized by physostigmine (7,79,93,183,216,288) and other cholinesterase inhibitors. Moreover, the beneficial effects of physostigmine are not shared by neostigmine, an AChE in-

served on acute dosing, it is unlikely that an explanation is afforded by tolerance development. Rather, the activation of presynaptic muscarinic receptors (inhibitory) is a more plausible explanation.

The hippocampus plays a critical role in learning and memory events, and the phenomenon of long-term potentiation (LTP) is widely recognized to be a valuable paradigm for the memory process (34,44). Physostigmine may induce phenomena similar to long-term potentiation by AChE inhibition, through potentiation of acetylcholine release, including antagonist actions at voltage-gated K^+ channels (114), or by direct cholinomimetic actions to provide a transient increase in excitability and subsequent block of GABAergic inhibitory transmission. This process may involve block of GABA release.

Cholinergic transmission may be impaired in aging animals, which may serve as models for physostigmine amelioration of memory deficits (101). In comparing 5- and 23-month-old Fischer 344 rats in a T-maze, the working memory retention of the 23-month-old rats was impaired compared with 5-month-old rats when tested with inter-run intervals, which is consistent with a decline in spatial learning with age (197). In this study, physostigmine (0.1 and 0.2 mg/kg) improved the age-related decline of memory, but did not significantly improve the performance of the young rats. An age-related decline is apparent in mealtime-associated activity rhythms that suggests impairment of temporal learning (235). Both the muscarinic agonist arecoline and physostigmine reduced this impairment in a dose-related manner in 24-month-old Wistar rats (198).

Physostigmine has also been examined for its acute effects on memory acquisition in young and aged primates (23,25,88). In rhesus monkeys (4 to 7 years and > 18 years), with physostigmine at doses of 0.0013 to 0.04 mg/kg, the effects on recent memory acquisition were absent at low doses, apparent at intermediate doses of 0.01 to 0.02 mg/kg and were detrimental at the highest doses of 0.04 mg/kg. Aged monkeys behaved similarly, but their responses were much more variable (88). In aged (18 to 26 years of age) and test-sophisticated Cebus monkeys physostigmine, employed under individualized best-dose conditions, revealed dose- and individual-dependent improvement in recent memory acquisition (23). In a small group (7 individuals) of young rhesus monkeys (4 to 7 years) the acute effects of physostigmine on an operant test battery were examined over the dose range 0.001 to 0.056 mg/kg (88). Physostigmine facilitated acquisition of simple spatial tasks at low doses, but the acquisition of more complex tasks was not affected or was impaired by physostigmine. The impaired operant test battery performance was concluded to originate from an action of physostigmine on the response rate rather than on cognitive process impairment.

A similar dose-dependency of physostigmine action has also been reported in monkeys with lesions in the nucleus basalis of Meynert (1,2). The responses of the aged, impaired primates to physostigmine were affected by the same general range of physostigmine concentrations, but the responses were far more variable. A comparison of physostigmine and other cholinomimetics in reversing scopolamine-induced deficits or improving visual recognition memory revealed physostigmine to be effective in both systems and to be superior to the other cholinomimetics studied, which included nicotine, pilocarpine, and arecoline (213,214). Since these two cognitive tasks of ac-

Fig. 10. *A.* Effects of continuous infusion of physostigmine (PHY) and oxotremorine (OXO) on motor habituation deficit in rats with basal forebrain lesions (BF). Spontaneous motor activity (SMA) and rearing counts were measured for 60 min. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with sham operated control, $P < 0.05$, $P < 0.01$ compared with BF control. *B.* Effects on passive avoidance deficits in basal forebrain-lesioned (BF) animals. Mean latencies in retention tests carried out 1, 4, and 24 h after training $^{*}P < 0.05$, $^{*\dagger}P < 0.01$ compared with sham operated control, $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ compared with BF control.

Fig. 10. *C*. Effects on the Morris water task deficit in basal forebrain-lesioned animals. Each rat received two blocks of four trials per day for 3 d. Each value represents the mean latency to escape on to the submerged platform. $^{*}P < 0.01$ compared with BF control. Reproduced with permission from reference 178, Miller SA, Blick DW, Kerenyi SZ, Murphy MR. Efficacy of physostigmine as a pretreatment for organophosphate poisoning. *Pharmacol Biochem Behav* 1993;44:343–347.

tive and passive learning differ in many important respects, it is of interest that both are approximately equally sensitive to the cholinergic stimulants employed and that physostigmine is probably the most effective in both (58,124). This is consistent with a comparison of physostigmine and 3,4-diaminopyridine (3,4-DAP) in aged Cebus monkeys, where physostigmine was also the more effective agent in the reduction of memory impairments (25).

The beneficial effects of physostigmine may be enhanced in combination with adrenergic stimulation. A combination of clonidine (which alone increases memory performance in primates) and physostigmine was more beneficial in a delayed matching-to-sample task than either agent alone (46,259). Part of this enhancement may be derived from the ability to use higher doses of physostigmine in the presence of clonidine.

SECONDARY ACTIONS

As a direct consequence of its actions on AChE and increased availability of acetylcholine, physostigmine has direct effects in a variety of organ and tissue systems,

including the cardiovascular system, smooth muscle, and systems where nicotinic receptors are present. Additionally, physostigmine has been shown to have actions or other systems-including nerve growth factors and amyloid protein processing. The relationship of these latter actions to the memory-enhancing properties of physostigmine remains to be determined.

Effects on the Cardiovascular System

The effects of physostigmine on the cardiovascular system are dependent upon the route and site of administration, central versus peripheral nervous system involvement, the species evaluated, and the anesthetic state. The general effects of acetylcholine on the vasculature are to induce vasodilatation through interaction at muscarinic receptors on endothelial cells and to promote bradycardia through an action on atrial pacemaker cells. It is increasingly clear, however, that central actions of physostigmine are particularly important in determining its cardiovascular profile (37–40,136,258). The early pharmacological literature ascribed the pressor effect of physostigmine in the dog to a central locus (39), a finding subsequently extended to the cat and rat (reviewed in 37–40). The pressor effect is due to a central cholinergically mediated activation of peripheral sympathetic responses. Generally, the concentrations of physostigmine needed to produce vasopressor responses and bradycardia are significantly less (by 5- to 10-fold) when the drug is administered centrally rather than peripherally. Although pressor responses to physostigmine appear in both conscious and anesthetized rats, the bradycardia seen in the anesthetized rat following intravenous or intracerebroventricular administration of physostigmine is converted to tachycardia in urethan anesthesia (50,122). The pressor effects of physostigmine in rats are dose dependent over the range of 50 to 150 μg/kg, increasing by 7 and 45 mm Hg at the lowest and highest doses, respectively (40,41). These pressor effects are blocked by atropine and by the selective muscarinic M_2 antagonist 4-diphenylacetoxy-N-methylpiperidine (4-DAMP) (Fig. 11) (40).

The cardiovascular effects of physostigmine are dependent upon central acetylcholine, since they are blocked by the prior administration of hemicholinium-3 (HC-3), which blocks choline uptake into nerve terminals (Table 14) (41,272). Stamenovic and Varagic (249) showed that the effect of physostigmine on centrally evoked sympathetic discharge in the rat was dose dependent with a good correlation between the duration of the pressor effect and preganglionic neural facilitation.

The physostigmine-evoked rise in sympathetic discharge is blocked by atropine, but not by the quaternary ammonium derivative methylatropine. That a significant component of the peripheral responses mediated by physostigmine is mediated through an increased activity of the central sympathetic component of the autonomic nervous system is revealed by a number of observations, including the rise in renin activity accompanying the presser response (4) and the lipolytic and glycogenolytic responses to physostigmine (263,270), responses that are absent with the peripheral AChE inhibitor neostigmine. Additionally, ganglion-blocking agents, central sympatholytic agents, and central sympathetic lesions also block physostigmine responses (reviewed in 38). A link of physostigmine's action on cardiovascular responses to the

opioid pathway is indicated by the naloxone-reversible effect of leu-enkephalin, met-enkephalin and β-endorphin in blocking the pressor response to physostigmine in the rat (271).

Regional cerebral blood flow increases following physostigmine administration, with accompanying changes in O_2 consumption (69,89,119,186). These studies suggest that there is a relationship between cerebral blood flow and brain metabolism that is maintained after oral administration of physostigmine. In humans, it is known that regional cerebral blood flow and cerebral metabolism are closely coupled events and this relationship parallels regional glucose metabolism (87). Clinical studies using single photon emission tomography show that physostigmine increases cerebral blood flow (124,262). Hunter et al. (124) described regional cerebral blood flow increasing in the left cortex relative to right with the greatest effect in the left frontal regions following administration of physostigmine (0.375 to 0.500 mg/kg). Tune et al. (262) reported in a limited study, however, that although physostigmine did increase blood flow in most patients, only one Alzheimer patient showed clinical improvement. This patient did show a major increase in cerebral glucose metabolism. Geaney et al. (92) reported that Alzheimer patients had reduced regional cerebral blood flow in the posterior parietotemporal region relative to controls and that this deficit was reduced following physostigmine administration, although changes in cognitive performance were not reported.

Other Centrally Mediated Effects

A number of other pharmacologic effects of physostigmine are also mediated through central mechanisms, including hypothermia, endocrine and neuroendocrine changes, and yawning. Physostigmine produces hypothermia in mice and rats (90,270), probably through activation of muscarinic receptors in the anterior hypothalamus (159). This dose-dependent response is not mimicked by neostigmine and is blocked by atropine, but not by methylatropine, consistent with a central locus of action. This hypothermic response is lost following downregulation of muscarinic receptors (58). A loss of temperature regulation and of endurance has been reported in rats exercised on a treadmill following physostigmine administration (167,168). These effects are dose-dependent and are accompanied by corresponding dose-related cholinergic phenomena, including salivation, tremors and defecation. Both endocrine and neuroendocrine responses occur following physostigmine administration. Lordosis in ovariectomized, estrogen-primed female rats is facilitated by eserine in an atropine-sensitive manner (57).

The cholinergic system has a defined role in the neuroendocrine axis (167). An important component of this is the release of corticotropin-releasing factor from the hypothalamus by acetylcholine, which has been established both *in vitro* and *in vivo*. Physostigmine in humans has been shown to elevate the levels of plasma cortisol, prolactin, arginine vasopressin, and growth hormone (34,115,208). The ability of scopolamine, but not methylscopolamine, to blunt these responses and the inability of neostigmine to mimic the actions of physostigmine is consistent with a central mode of action of physostigmine (34,57,115). A good correlation was observed between

plasma ChE inhibition and cortisol levels (115, 146). Stimulants such as amphetamine and methylphenidate cause a rise in plasma cortisol levels when administered to normal patients, but this response is blunted in endogenous depression (219). This effect of depression can be mimicked by physostigmine in normal patients (134). In both rhesus monkeys and humans, the levels of the vasoconstrictor and cardiodepressant neuropeptide Y are elevated in CSF by approximately 50% above baseline, from 15 to 45 min following intravenous administration of physostigmine 15 μg/kg given in the presence of the peripheral muscarinic blocker glycopyrrolate (29). Age-related influences were not determined.

The cholinergic system is involved in the central mediation of yawning (266,267). Physostigmine, but not neostigmine, induces yawning in rats, an effect which is mimicked by dopamine D_2 agonists and D_1 antagonists (269,289,290). The teeth-chattering response induced by physostigmine in rats is blocked by mecamylamine and may represent a nicotinic-type response (268).

Peripherally Mediated Responses

The dominant peripheral effects of physostigmine, outside of the cardiovascular system, are exerted on the gastrointestinal, genitourinary, and respiratory tracts and secretory cells. These effects, mediated through indirect stimulation of muscarinic receptors in the smooth muscle cells and indirect stimulation of both muscarinic and nicotinic receptors in ganglia, lead to a tension response in smooth muscle and enhanced glandular secretion. These actions contribute to the excess cholinergic stimulation seen in cholinergic crisis. The contracture of bronchial musculature by physostigmine has long been known (77,261) and is undoubtedly peripheral in origin since it can be observed in isolated preparations. Intestinal smooth muscle is similarly sensitive to physostigmine and in the intact animal it produces increased peristalsis and strong rhythmic contractions (45,112,220). These effects were shared by other cholinesterase inhibitors, including tetra-ethylpyrophosphate, neostigmine, diisopropylfluorophosphonate, and hexaethyltetraphosphate. Circular muscle may be more sensitive than longitudinal muscle to cholinesterase inhibition (112), perhaps because of the higher concentration of enzyme in the former tissue (233,234).

Actions at Nicotinic Receptors

There is increasing evidence that stimulation of neuronal nicotinic acetylcholine receptors enhances cognitive processes, which may be of potential value in Alzheimer's therapy (13,253). The physiological transmitter acetylcholine is an agonist at both muscarinic and nicotinic receptors in the CNS. Therefore, it is of potential therapeutic importance that physostigmine appears to have direct actions at neuronal nicotinic receptors. Modulators of nicotinic receptors are of interest because nicotine is an anxiolytic and has neuroprotective and cognition-enhancing properties (13). Physostigmine exerts stimulating, desensitizing, and blocking actions at nicotinic receptors in addition to its AChE-inhibitory actions at the neuromuscular junction. The agonist ef-

fects of physostigmine were observed as early as 1977 (137) and confirmed a number of times (11,202,203,228,233,234). Further support for these actions additional to AChE inhibition, stems from observations that (+) physostigmine, which is virtually devoid of AChE-inhibitory activity, is an agonist at nicotinic acetylcholine receptors (11). Photoaffinity labeling of nicotinic acetylcholine receptors by (+) physostigmine has revealed a pharmacologically distinct drug binding site carried on the same subunit as that for acetylcholine (246). The presence of a specific binding site was also reported by Schrattenholtz et al. (228) who characterized a high affinity binding site, K_D = 35 nM, for the competitive physostigmine analog 1-methylphysostigmine.

These effects of physostigmine at the nicotinic receptor site are exerted at concentrations comparable to those that inhibit cholinesterase: in frog skeletal muscle concentrations of physostigmine from 1 to 200 μM induced channel opening and served to bind to and ultimately block the open state of the channel, thus providing both agonist and antagonist effects. Similarly, the carbamate analogs pyridostigmine and neostigmine also exhibited agonist effects at the receptor, but at concentrations significantly higher than those needed to block AChE (234). In cultured hippocampal neurons, physostigmine at concentrations of 1 to 10 μM activated nicotinic receptors and similar activation was found in studies of ion currents in vesicles from Torpedo electroplax (233). Physostigmine at concentrations of 0.3 to 300 μM blocked the stimulant effect of nicotine on dopamine release in rat striatal synaptosomes (56). These actions may reflect a partial agonist effect of physostigmine at nicotine receptors. It is possible that such receptor interactions may underlie the ACh releasing effects of physostigmine reported in a variety of preparations, including cat brain (107), guinea pig atria (21), rat brain (278), guinea pig ileum (63), and human brain slices (124,192,193). Actions at both muscarinic and nicotinic receptors may be involved. The releasing effect of physostigmine is not shared by all cholinesterase inhibitors; in guinea pig ileum the potent irreversible inhibitor mipafox (diisopropyl-phosphodiamidic fluoride) has only slight effects on acetylcholine release (63). In guinea pig trachealis muscle, both physostigmine and neostigmine produced atropine-sensitive contractions after treatment of the smooth muscle with mipafox (51). However, only nonsurmountable antagonist effects of physostigmine, 0.3 to 300 μM, were observed in dopamine release in a rat striatum preparation (56).

Actions at c-AMP Phosphodiesterase

Physostigmine, but not neostigmine, inhibits c-AMP phosphodiesterase with a K_i value of 18 μM for the high-affinity form, and is approximately ten times more potent than theophylline (68). This activity may underlie some effects of physostigmine on nerve terminals by promoting c-AMP-dependent events.

Actions on Amyloid Protein Processing

There is strong evidence that the abnormal processing of amyloid precursor protein (APP) to yield the insoluble and neurotoxic β-amyloid peptide (β-A4) may be critical

to the deposition of the neuritic plaques that are characteristic of Alzheimer pathology and neuronal dysfunction (reviewed in 14,61,231). However, the specific signal(s) that trigger this abnormality of amyloid processing is(are) not well defined. Some evidence suggests a neurochemical link to amyloid processing and, in particular, a role for cholinergic innervation. It was early recognized that a common characteristic shared by the several neuronal groups demonstrating Alzheimer pathology was the presence of AChE (24,64,173,184). This hypothesis is of particular interest since it links the two current major hypotheses of Alzheimer dysfunction — neuronal acetylcholine loss and amyloid deposition — and provides additional rationale for cholinergic replacement therapy.

Non-neuronal cells (human embryonic kidney cells) transfected with the genes for the human M_1 and M_3 muscarinic receptors, showed a rapid and significantly increased release of soluble APP derivatives by receptor stimulation through a protein kinase C-dependent pathway (49,195). Of related interest is the loss of subcortical innervation in the cerebral cortex which is accompanied by a rapid and persistent increase in the levels of β-APP mRNA levels (275). Thus, lesions of the cholinergic nucleus basalis of Meynert elevates the *ex vivo* synthesis of this precursor protein. Consistent with this work, cholinesterase inhibitors have now been shown to increase the secretion of APPs in rat brain cortex (180). Physostigmine at concentrations of 0.01, 0.1, and 1.0 μM resulted in 5%, 25%, and 64% inhibition, respectively, of AChE in the brain slice, which resulted in a significant enhancement (148% of basal) of APP release at the 0.1 μM concentration.

Bovine brain AChE and the human and mouse recombinant enzymes accelerate amyloid formation from the amyloid 1-40 peptide in *in vivo* preparations (128). This activity of AChE was independent of the active site since amyloid formation was not affected by active site inhibition; however, the peripheral anionic site was involved since amyloid formation was blocked by the presence of the peripheral site antagonist propidium. BuChE does not exhibit this property.

Actions at Nerve Growth Factor Receptors

Nerve growth factor (NGF) is a neurotrophic agent critical to the development and survival of neurons and to the establishment of neuronal connections (152). Nerve growth factors may have a role in the treatment of neurodegenerative disorders (153). Cerebellar Purkinje neuron survival in culture was enhanced by the simultaneous presence of both carbachol and nerve growth factor; neither agent was effective alone (181). These observations suggest a role for cholinergic innervation in regulating the level of NGF and NGF receptors and in the maintenance of neuronal integrity. Chronic physostigmine treatment increases NGF receptor density in neuronal tissue (3). Physostigmine infusion at 1 mg/kg increased the density of high- and low-affinity binding sites at NGF receptors in rat brain by 3- and 6-fold, respectively, while acutely decreasing NGF content. These affects on NGF content were transient and returned to control levels after 7 d. The relationship of these effects to long-term actions of physostigmine is unknown.

BIOCHEMICAL CONSEQUENCES OF PHYSOSTIGMINE ACTION

In neuronal systems many actions of acetylcholine at the muscarinic receptors involve stimulation of phosphatidylinositol turnover and the generation of inositol polyphosphates, notably inositol 1,4,5-trisphosphate (IP_3), and acylglycerols as second messengers (30,31,85). Inositol trisphosphate has a major role in intracellular Ca^{2+} signaling, serving to release Ca^{2+} to support Ca^{2+}-dependent stimulus-response coupling via intracellular IP_3 receptors (85). Similarly, the acylglycerols serve as activators of protein kinase C, a key kinase regulating the activities of a variety of cellular effectors including ion channels (85,194).

These effects are mimicked or amplified by physostigmine in various regions of the rat brain, including cerebral cortex (139,147), striatum (100), and hippocampus (285). The effects of physostigmine are region-specific, being greatest in the cortex and least in the cerebellum, and parallel the distribution of muscarinic receptors (210). Similarly, cholinergic agonists increase tissue levels of c-GMP through muscarinic receptor stimulation of guanylyl cyclase (54); these effects are mimicked by physostigmine (99,121). c-GMP plays a number of messenger roles and is also an intermediate in the activation of nitric oxide by soluble guanylate cyclase (91,126,140). c-GMP activates c-GMP-dependent protein kinase, and c-GMP-dependent phosphodiesterase, and serves to control ADP-ribosyl cyclase-dependent Ca^{2+} release.

Receptor Regulation by Physostigmine

A consequence of prolonged occupancy of muscarinic receptors by agonist or antagonist is receptor down- or up-regulation, respectively (232). Agonist-promoted down-regulation (and desensitization) will reduce the apparent efficacy of an agonist. Apparently physostigmine does not produce major down-regulation when administered either by continuous infusion or discontinuous administration (33,200,205). Although constant infusion of physostigmine in guinea pigs for 7 d (via implanted osmotic mini-pumps) at rates of 0.12 and 0.24 mg/kg/h did produce marginal muscarinic receptor down-regulation of 10% and 15%, respectively, tolerance did not develop to a high dose of physostigmine as measured by effects on body weight, water consumption, and body temperature (98).

In contrast, tolerance to the effects of continuous DFP administration does occur, as well as large reductions (down-regulation) in acetylcholine receptor number (62,150,217,218,287). These differences probably relate to the shorter half-life of physostigmine and the more rapid hydrolysis of the carbamylated AChE relative to the phosphorylated AChE yielding a less persistent occupancy of the enzyme.

It is not known whether physostigmine produces any differential effects on muscarinic receptor subtypes. Persistent muscarinic receptor stimulation may also promote neuronal survival: in cultured Purkinje cells physostigmine produced an increase in neuron survival that was further enhanced in the presence of NGF (181). Despite the relative absence of effects of physostigmine on muscarinic receptors, regulatory ac-

tions on other receptor systems have been described that may be of potential importance. Mice tolerant to physostigmine showed an increased amount of nicotine binding in several brain regions (33). Rats discontinuously treated with physostigmine for 13 d showed an increased release of acetylcholine from hippocampal slices and an approximate doubling of nicotine binding in the same brain region (72). Nicotine has neuroprotective, anxiolytic, and cognition-enhancing actions in both animals and humans (13).

NGF receptors were increased in rat brain infused with physostigmine (1 mg/kg/d), but this expression was transient and the significance unknown (3). However, NGF does have neuroprotective properties and it is possible that some part of any therapeutic benefit of physostigmine in Alzheimer's brain may stem from these properties.

POSSIBLE ADVERSE EFFECTS

The dominant pharmacology of physostigmine is that of an indirect cholinergic stimulant at both central and peripheral sites of the nervous system (172,258,291). These actions determine the therapeutic roles for physostigmine and contribute to the general potential adverse reactions (Table 15) (207,277). Thus, physostigmine is potentially capable of producing effects equivalent to excessive stimulation of both cholinergic and nicotinic receptors through the central and peripheral nervous systems. A cholinergic crisis (172,207,258,277) may result from physostigmine overdose. Cholinergic crisis is characterized by excessive sweating, salivation, nausea, vomiting, sinus bradycardia, hypotension, muscle weakness, and paralysis. Death can result from respiratory muscle paralysis.

The pharmacology of physostigmine is predominantly that of acetylcholine action at central and peripheral muscarinic and nicotinic cholinergic receptors. This pharmacology also dominates the drug interaction profile of physostigmine. These interactions have been documented in standard reference works (207,277,291). Accordingly,

TABLE 15. Adverse reactions to physostigmine attributable to cholinergic overstimulation

Abdominal pain	Myasthenia
Blurred vision	Myopia
Bronchospasm	Nausea/Vomiting
Ciliary spasm	Ocular irritation
Diaphoresis	Ocular pain
Dyspnea	Photophobia
Headache	Retinal detachment
Increased intraocular pressure	Salivation

physostigmine will counteract the peripheral and central effects of atropine and related parasympatholytic agents. These effects will be reciprocal, but the precise balance will depend on the relative central and peripheral effects of the parasympatholytic agent. Similarly, physostigmine will be expected to have additive effects with cholinergic agonists which may produce a cholinergic crisis of drug overdose. Physostigmine inhibits the hydrolysis by plasma cholinesterase of depolarizing neuromuscular blocking agents such as succinylcholine and will, therefore, prolong the neuromuscular paralysis induced by these agents. In contrast, physostigmine will competitively overcome the actions of nondepolarizing neuromuscular blocking agents and will reduce the neuromuscular paralysis induced by these agents (47).

Physostigmine has been considered by many to be a useful antidote to overdoses of tricyclic antidepressants. Although, physostigmine does not reduce the non-cholinergic quinidine-like effects of these antidepressants, it may reduce the duration of coma. The use of physostigmine as an antidote may be misleading and it is no longer recommended. In the cardiovascular system, physostigmine is a hypotensive and bradycardic agent. It is likely that some caution is to be employed for physostigmine use in the presence of cardiovascular drugs, notably those that produce bradycardia including β-blockers and Ca^{2+} antagonists (specifically verapamil and diltiazem). Additionally, some data suggest that hypotension may be a factor contributing to a reduced cognition-enhancing activity of physostigmine (223). The muscarinic effects of physostigmine are stimulant on nonvascular smooth muscle including the gastrointestinal and genitourinary tracts and the bronchioles. Accordingly, there will be a general contraindication for physostigmine in the presence of gastrointestinal obstruction, peptic ulcer, urinary tract obstruction, and asthma. Additionally, the stimulant effects of physostigmine on these several smooth muscle systems may interact with drugs used to reduce nonvascular smooth muscle tone, including β-agonists in respiratory smooth muscle. Finally, physostigmine has been shown experimentally to reduce ethanol sedation in mice (84,209). The clinical significance of this effect is not known, nor is it known whether alcohol diminishes the cognition-enhancing actions of physostigmine.

CONCLUSIONS

The central and peripheral pharmacology of physostigmine is predominantly that of acetylcholine, the transmitter at cholinergic synapses. As a tertiary, amine, physostigmine can penetrate the blood-brain barrier. Several studies have documented its ability to increase the levels of acetylcholine centrally in a regionally selective and dose-dependent fashion. Such selectivity depends, in part, on the level of cholinergic activity at different synapses and brain areas. Major pharmacological effects of physostigmine are exerted at central cholinergic synapses. Rodent and primate models have demonstrated that physostigmine has efficacy in behavioral, memory, and learning models. Deficits in such models can be produced by anticholinergic drugs, including scopolamine, and these are overcome by physostigmine. These effects of physostigmine are centrally mediated and not attributable to any peripheral actions. The effects of physostigmine on primate models of memory and memory acquisition, including those

of aging animals, are dose-dependent and are reported to be more potent than those of other cholinesterase inhibitors.

The peripheral effects of physostigmine may be complex, since they can be exerted at both ganglionic and post-ganglionic sites in the sympathetic and parasympathetic divisions of the autonomic nervous system. In the cardiovascular system, physostigmine produces pressor actions with bradycardia; these effects likely involve a significant central component. Because of its lack of cholinergic innervation, vascular smooth muscle is not directly sensitive to physostigmine. The smooth muscle of the viscera, including the gastrointestinal and urinary tracts, is sensitive, however, and responds with increased contractile and secretory activities. Accompanying the enhanced gastrointestinal motility are enhanced secretory responses of both enzymes and acid production. Similarly, the cholinergically sensitive sweat and salivary glands respond to physostigmine with increased secretion. These responses are sensitive to peripherally acting anticholinergic drugs.

Although the pharmacology of physostigmine is dominated by its actions on AChE some secondary pharmacological effects have been described, including direct actions at the ion channel component of nicotinic receptors, actions at acetylcholine release sites of nerve terminals, and phosphodiesterase-inhibitory activity. Additionally, physostigmine has a regulatory effect on a number of neurotransmitter receptors, including those for acetylcholine, that may contribute to its long-term effects on cholinergic function.

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