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Pharmacology of selective acetylcholinesterase inhibitors: implications for use in Alzheimer's disease

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Abstract

Cholinesterase inhibitors vary in their selectivity for acetylcholinesterase versus butyrylcholinesterase. We examined several cholinesterase inhibitors and assessed the relative role of acetylcholinesterase versus butyrylcholinesterase inhibition in central and peripheral responses to these medications. Donepezil and icopezil are highly selective for acetylcholinesterase, whereas tacrine and heptylphysostigmine demonstrated greater potency for butyrylcholinesterase over acetylcholinesterase. All four compounds increased acetylcholine levels in mouse brains. Dose–response curves for tremor (central effect) and salivation (peripheral effect) showed that donepezil and icopezil possess a more favourable therapeutic index than the nonselective inhibitors, tacrine and heptylphysostigmine. Co-administration of the selective butyrylcholinesterase inhibitor tetraisopropylpyrophosphoramide (iso-OMPA) potentiated peripheral, but not central, effects of the selective acetylcholinesterase inhibitor icopezil. The improved therapeutic index observed in mice with icopezil is due to a high degree of selectivity for acetylcholinesterase versus butyrylcholinesterase, suggesting that high selectivity for acetylcholinesterase may contribute to the clinically favourable tolerability profile of agents such as donepezil in Alzheimer's disease patients.

Keywords: Alzheimer's disease; Cholinesterase inhibitor; Acetylcholinesterase; Butyrylcholinesterase

1. Introduction

The cognitive symptoms that characterize Alzheimer's disease are thought to be related to the degeneration of cholinergic neurons in the cerebral cortex and subcortical structures (Davies and Maloney, 1976). A number of preclinical studies have suggested an association between the cholinergic system and cognition (Flicker et al., 1983; Brito et al., 1983; Ingram, 1988; Beeri et al., 1995; M'Harzi et al., 1995; Pavone et al., 1993). For example, experimental lesions of the basal forebrain cholinergic system (Flicker et al., 1983) and treatment of animals with muscarinic antagonists (Brito et al., 1983; Ingram, 1988) produce memory deficits. Overexpression of acetylcholinesterase in transgenic mice has been shown to lead to a progressive cognitive deficit (Beeri et al., 1995). Conversely, cholinergic stimulation can enhance performance of cognitive tasks both in animals and in man (Rusted and Warburton, 1992; Pavone et al., 1993). These observations are consistent with a critical role for acetylcholine in cognitive function and suggest that replacement therapy with cholinomimetic agents may improve the cognitive and memory deficits characteristic of Alzheimer's disease.

Cholinesterase inhibitors are the only class of compounds to date that have consistently proven to be efficacious in treating the cognitive and functional symptoms of Alzheimer's disease (Weinstock, 1999). Cholinesterase inhibitors represent the cornerstone of therapy for Alzheimer's disease, and four of these medications have been approved for the symptomatic treatment of mild to moderate Alzheimer's disease. These are tacrine (an aminoacridine), donepezil (a benzylpiperidine), rivastigmine (a carbamate) and galantamine (a tertiary alkaloid). Since these compounds appear similar in efficacy (Weinstock, 1999; Wilkinson et al., 2002), their clinical differentiation may rely on differences in tolerability profiles and ease of use. Differences in the

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Table 1
In vitro inhibition (IC_{50}[a]) of enzymes involved in acetylcholine metabolism

Enzyme	Donepezil	Icopezil	Tacrine	Heptylphysostigmine	Iso-OMPA
Acetylcholinesterase (nM)	19±7	0.33±0.09	108±7	110±20	>100,000
Butyrylcholinesterase (nM)	4100±1800	7200±1200	29±2	17	1200
HACU (μM)	>100	>50	>100	ND	ND
ChAT (μM)	>100	>10	>100	ND	ND

ND=not determined; HACU=high affinity choline uptake; ChAT=choline acetyltransferase.
[a] IC_{50}=Concentration producing 50% inhibition.

tolerability profiles of cholinesterase inhibitors may arise as a result of differential selectivity for acetylcholinesterase versus butyrylcholinesterase (Rogers et al., 1998; Rogers and Friedhoff, 1998). There is some evidence to suggest that butyrylcholinesterase activity may be involved in the pathogenesis of Alzheimer's disease (Mesulam and Asuncion Moran, 1987). This has led to the hypothesis that the use of nonselective cholinesterase inhibitors that inhibit both butyrylcholinesterase and acetylcholinesterase may be more beneficial to patients with Alzheimer's disease than the use of selective cholinesterase inhibitors that inhibit acetylcholinesterase alone (Weinstock, 1999).

Experiments were conducted to compare the pharmacology of three classes of cholinesterase inhibitors: the aminoacridines (e.g. tacrine), carbamates (e.g. heptylphysostigmine) and benzylpiperidines (e.g. donepezil and icopezil). Assessments of the relative importance of acetylcholinesterase versus butyrylcholinesterase inhibition in centrally (i.e. brain acetylcholine levels and tremor) and peripherally (i.e. salivation) mediated cholinergic responses to cholinesterase inhibitors were undertaken. This was achieved using the selective butyrylcholinesterase inhibitor, tetraisopropylpyrophosphoramide (iso-OMPA) in combination with a selective acetylcholinesterase inhibitor and a nonselective acetylcholinesterase/butyrylcholinesterase inhibitor.

2. Materials and methods

2.1. Drugs and animals used

Male Sprague–Dawley rats (160–250 g; Charles River) were housed in hanging wire cages and male CD mice (20–25 g; Charles River) were housed in shoe box cages. All animals were allowed free access to food and water until overnight fasting prior to study. All in vivo experimental procedures were performed in compliance with the Laboratory Animal Welfare Act (1985) under protocols approved by the Pfizer Institutional Animal Care and Use Committee. Tacrine, physostigmine and iso-OMPA were purchased from Sigma Chemical Co. (St. Louis, MO). Donepezil and icopezil were synthesized at Pfizer. For oral administration, drugs were dissolved in distilled water. For subcutaneous delivery, iso-OMPA was prepared in 5% dimethylsulfoxide/95% saline.

Table 2
Effect of icopezil and tacrine on acetylcholine receptors in vitro[a]

Receptor	Icopezil	Tacrine
Muscarinic		
m1	1.2±0.3	0.7±0.03
m2	0.9±0.3	0.9±0.1
m3	2.0±1.1	1.1±0.1
m4	0.7±0.2	1.1±0.1
m5	2.4±1.7	6.7±0.5
Nicotinic	>10	>10

Binding assays were performed in triplicate. In addition to the assays shown, icopezil was found to be inactive (IC_{50}>1 μM) at the following receptors and enzymes: α-1-, α-2- and β-adrenergic; dopamine D1 and D2; 5-$HT1_A$, 5-$HT1_C$, 5-$HT1_D$, 5-HT2, 5-HT3; histamine H_1 and H_2; adenosine A_1 and A_2; $GABA_A$; benzodiazepine; μ opioid; tachykinin NK_1, NK_2 and NK_3; PCP; NMDA; NMDA (glycine site); AMPA; kainate; Ca^{2+} channels (verapamil site); Ca^{2+} channels (dyhydropyridine site); noradrenaline uptake; dopamine uptake; serotonin uptake; monoamine oxidase inhibition.
[a] All values are expressed as mean K_i in μM±S.D. of three independent determinations.

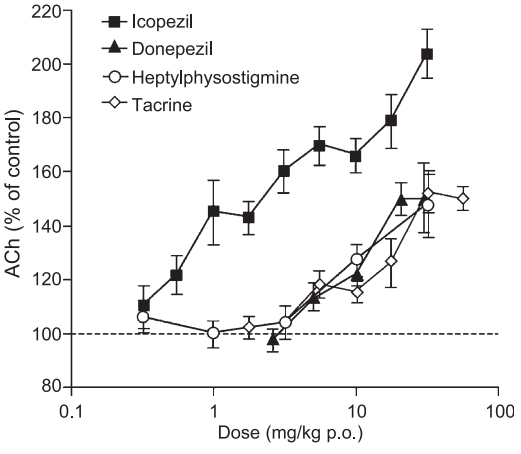

Fig. 1. The effects of icopezil, donepezil, heptylphysostigmine and tacrine on mouse brain acetylcholine following their oral administration. Acetylcholine levels are expressed as the percentage of control values (mice receiving the relevant vehicle only). Drugs were administered 1 h before sacrifice. The results are the mean±S.E.M. of 6–24 observations at each dose.

2.2. Cholinesterase assays

Human acetylcholinesterase (EC 3.1.1.7) purified from erythrocytes and human butyrylcholinesterase (EC 3.1.1.8) purified from serum were purchased from Sigma. Assays were performed essentially as described by Ellman et al. (1961) at 20–22 °C in 0.1 M sodium phosphate buffer (pH 8.0) containing 200 μM substrate (acetylthiocholine or butyrylthiocholine), 100 μM dithiobisnitrobenzoic acid, and 0.005 units enzyme in a final volume of 250 μl. Following a 15-min pre-incubation with inhibitor and enzyme, the reaction was started by the addition of substrate. Initial reaction rate was determined over 5 min by monitoring absorbance changes at 412 nm, and the percentage inhibition due to the presence of test compounds was calculated. For determination of kinetic parameters, initial reaction rates were measured at varying substrate concentrations (25–1600 μM) and the data were analysed using a non-linear least squares analysis.

2.3. Choline acetyltransferase

Activity of choline acetyltransferase (EC 2.3.1.6) was determined according to the method of Fonnum (1975). Rat forebrain homogenate was incubated with 8 mM choline (containing approximately 45,000 dpm ^{3}H-choline; New England Nuclear) and 200 μM acetyl-coenzyme A at pH 7.4 for 15 min at 37 °C. Following the addition of sodium tetraphenylboron (2 mg/ml in acetonitrile), labelled acetylcholine was extracted into a toluene-based scintillation cocktail and counted.

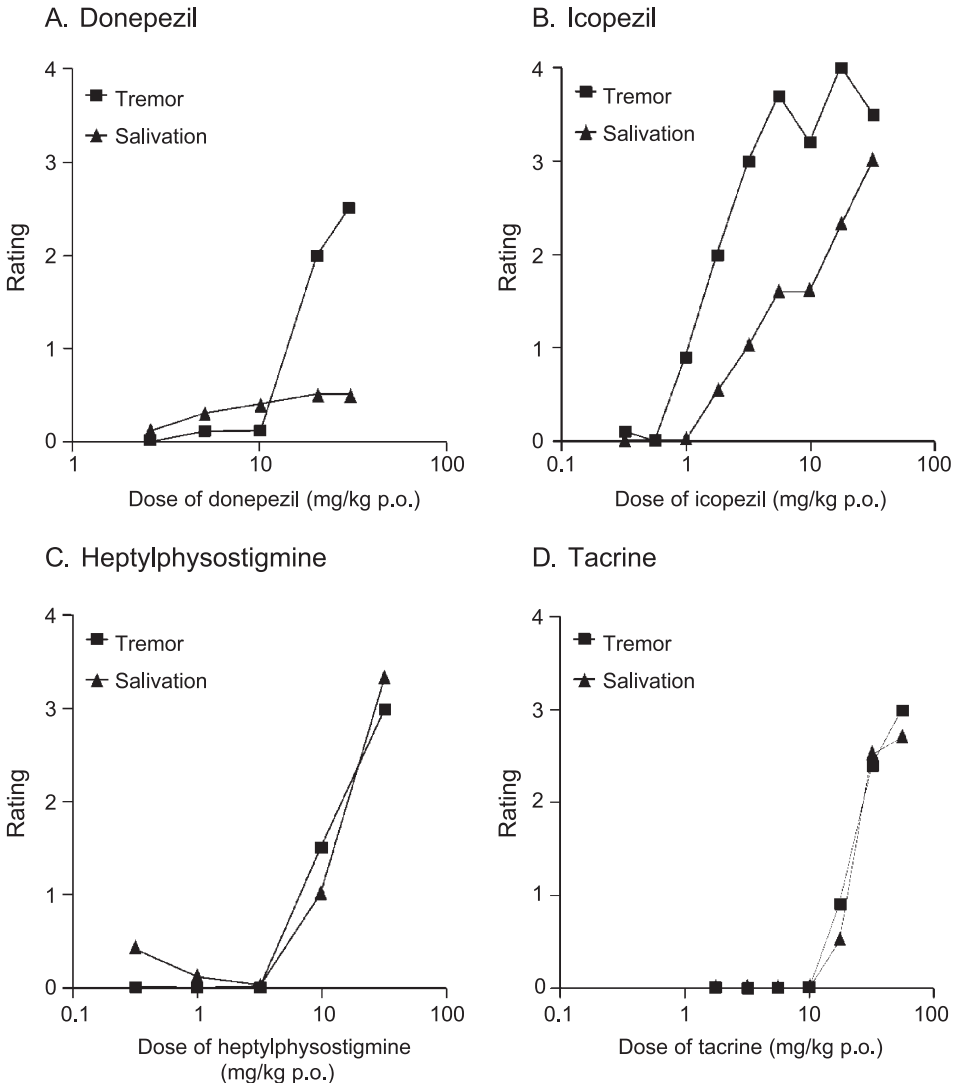

Fig. 2. The effects of (A) donepezil, (B) icopezil, (C) heptylphysostigmine and (D) tacrine on tremors and salivation in mice administered oral doses. Data were pooled from 1–3 experiments, in which n = 8 for each dose in each experiment. At higher doses of all compounds some of the animals died.

2.4. High affinity choline uptake

The high affinity choline uptake was evaluated using standard protocols (Yamamura and Snyder, 1972). Briefly, freshly prepared synaptosomes from rat cortex were incubated for 10–15 min at 37 °C in Tris-buffered Krebs solution with 100 μM choline containing $2-10\times10^5$ dpm tritiated choline. Accumulated substrate was separated by rapid filtration and quantified using liquid scintillation counting.

2.5. Acetylcholine concentration in rodent tissues

For determination of acetylcholine levels in rodent brains, animals were sacrificed by focused microwave irradiation of the head (2.8 s at 1.3 kW). Brains were homogenized in 20 mM sodium phosphate buffer (pH 5.3), and acetylcholine content in high-speed supernatants was determined using high-performance liquid chromatography with electrochemical detection of acetylcholine. Aliquots (10 μl) of tissue extract were injected onto a polymeric anion exchange column at a flow rate of 0.5 ml/min (50 mM sodium phosphate, pH 8.5) to resolve acetylcholine from choline. A post-column reactor containing immobilized acetylcholinesterase and choline oxidase enzymatically converted both acetylcholine and choline to betaine and hydrogen peroxide; the hydrogen peroxide was measured by electrochemical detection on a platinum-working electrode at +500 mV versus an Ag/AgCl reference electrode. Sensitivity was 0.1 pmol acetylcholine. Typical acetylcholine control values were 25–33 nmol/g in mouse forebrains. Data for drug-treated animals are reported as percent control values. In general, the treatment consisted of eight animals per group. Statistical significance was determined using Student's one-tailed *t*-test.

2.6. Ligand-binding assays

Receptor binding was performed by standard methods (Yamamura and Snyder, 1974). For nicotinic receptor assays, membranes were prepared from rat forebrain and incubated in buffer containing 50 mM Tris, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$ (pH 7.4) and ^{3}H-Nicotine (3 nM). For muscarinic receptor assays, Chinese hamster ovary cells expressing the cloned human m1–m5 muscarinic receptors (obtained from Dr. Tom Bonner, NIH) were used as the membrane source, and ^{3}H-*N*-methylscopolamine (0.5 nM) was used as ligand. Bound ligand was isolated by rapid filtration and quantified by liquid scintillation counting. Non-specific binding was defined by adding an excess of the unlabelled ligand.

2.7. General in vivo physiological measures

General observations of gross physiological and behavioural effects of standard and test compounds were made in male CD-1 mice (Charles River). Immediately after the oral administration of the test compounds, animals were placed individually in novel cages where tremors and salivation were rated subjectively. Previous studies have indicated that tremor is a useful measure of central cholinergic activation (Soncrant et al., 1985; Hallberg and Almgren, 1987; Sanchez and Meier, 1993). Conversely, the measurement of salivation is a useful measure of peripheral cholinergic activation (Domino and Corssen, 1967).

Tremors were rated on the following 5-point scale: 0=none; 1=slight evidence of tremors; 2=obvious tremors; 3=tremors most of the time; 4=extreme tremors and possible mortality. A 5-point scale was also used to rate salivation: 0=none; 1=salivation just observable; 2=obvious salivation; 3=marked salivation; 4=extreme salivation all over the mouth and forepaws. Ratings were made 15, 30 and 60 min after treatment; results at 60 min are reported. Doses were escalated until lethality was observed to provide an estimate of acute LD_{50}.

2.8. In vivo selectivity experiments

To ascertain the role of butyrylcholinesterase inhibition in the central (tremors and brain acetylcholine levels) and peripheral (salivation) responses to cholinesterase inhibitors, we conducted studies combining the highly selective, irreversible butyrylcholinesterase inhibitor, iso-OMPA, with the most selective acetylcholinesterase inhibitor (icopezil). The effects alone and in combination were compared with those of a nonselective cholinesterase inhibitor, tacrine. To determine the doses of iso-OMPA required to inhibit butyrylcholinesterase in vivo, mice were injected with iso-OMPA (2, 4, 8, 12 mg/kg, s.c.) and plasma was sampled 1 h later. All doses produced complete inhibition of plasma butyrylcholinesterase.

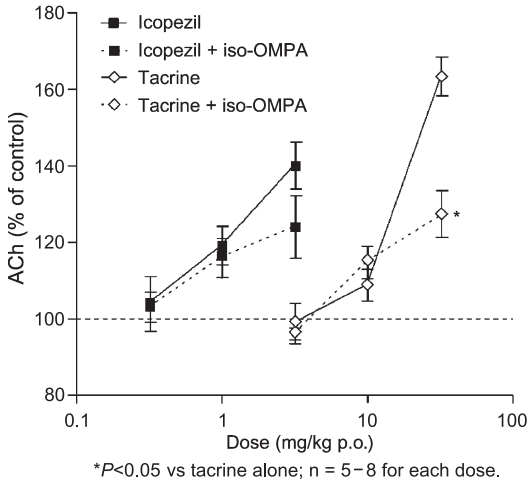

Fig. 3. The effects of the addition of iso-OMPA to the icopezil- and tacrine-associated effects on mouse brain acetylcholine levels. Data are expressed as mean±S.E.M.

3. Results

3.1. In vitro pharmacology of donepezil, icopezil, heptylphysostigmine and tacrine

Donepezil and icopezil were more potent inhibitors of acetylcholinesterase than butyrylcholinesterase, showing >200-fold selectivity for acetylcholinesterase versus butyrylcholinesterase (Table 1). In contrast, tacrine and heptylphysostigmine showed 3.7- and 6.5-fold greater potency for butyrylcholinesterase compared with acetylcholinesterase, respectively. In preparation for in vivo selectivity tests, icopezil and tacrine were profiled more broadly against a panel of central nervous system-relevant receptors and enzymes. Neither compound inhibited high affinity choline uptake or choline acetyltransferase, indicating no direct effect on acetylcholine biosynthesis. No competition at nicotinic receptors was observed up to 10 μM, although weak binding to some muscarinic receptors was detected (Table 2). Binding to a number of neurotransmitter receptors was negligible at 10 μM.

3.2. In vivo and ex vivo pharmacology

All of the cholinesterase inhibitors increased brain acetylcholine levels in mice in a dose-dependent manner (Fig. 1). Icopezil was the most potent and efficacious of the compounds tested, elevating mouse brain acetylcholine levels to twice those observed in controls at the highest dose (32 mg/kg). Donepezil, heptylphysostigmine and tacrine all increased acetylcholine levels by approximately 50% at the highest doses tested. The selective acetylcholine inhibitors, donepezil and icopezil, demonstrated a greater potency to induce a central effect (tremors) than peripherally mediated

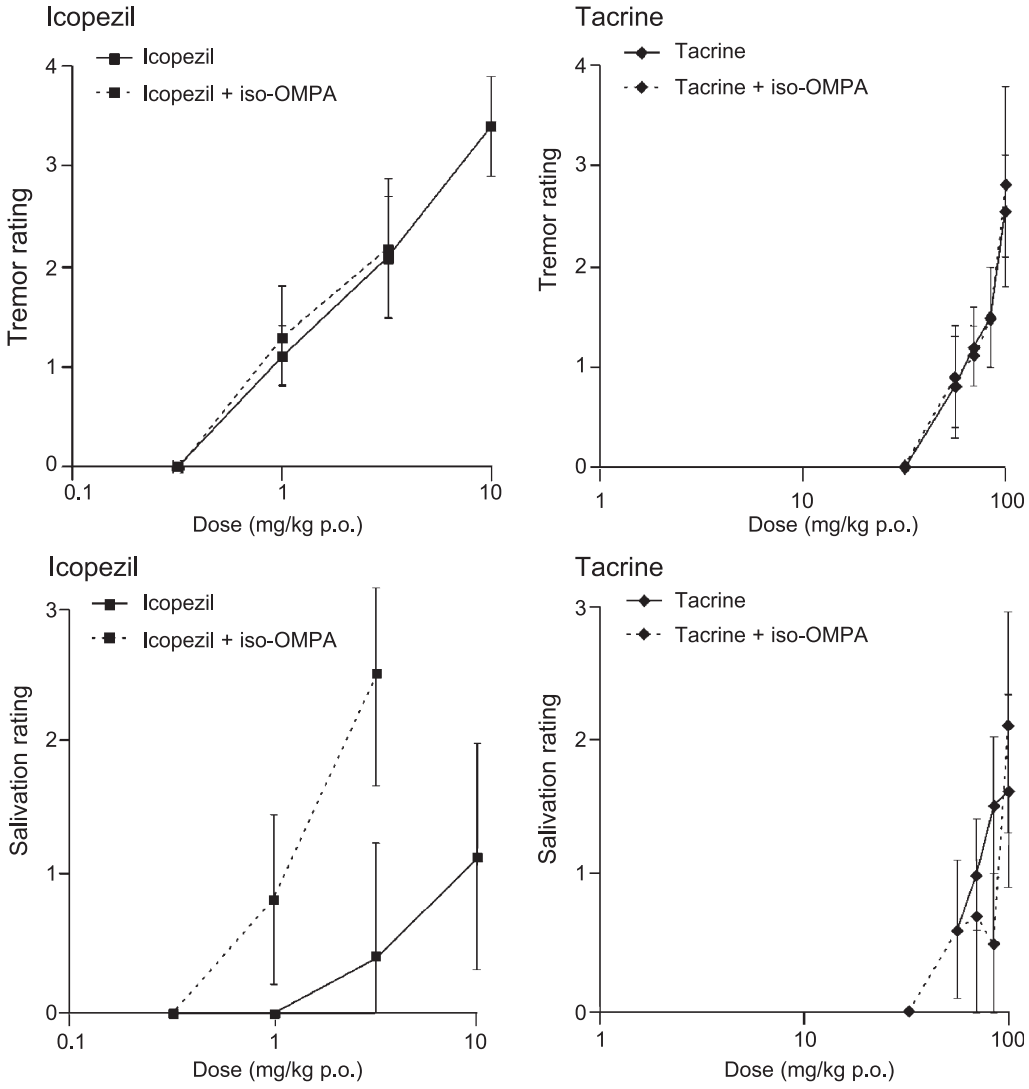

Fig. 4. The effects of the addition of iso-OMPA to the tremor- and salivation-inducing effects of icopezil and tacrine. Data are expressed as mean±S.E.M. Data were pooled from 2 experiments, in which n=9 for each dose.

salivation. In contrast, the nonselective cholinesterase inhibitors, tacrine and heptylphysostigmine, were equipotent at inducing tremor and salivation (Fig. 2A–D).

3.3. Effects of butyrylcholinesterase inhibition on physiological actions of cholinesterase inhibitors

To determine the relative importance of the inhibition of acetylcholinesterase versus butyrylcholinesterase to the central (tremors and brain acetylcholine levels) and peripheral (salivation) responses to cholinesterase inhibitors, the selective irreversible butyrylcholinesterase inhibitor, iso-OMPA, was administered in combination with icopezil, the compound most selective for acetylcholinesterase over butyrylcholinesterase, or with tacrine, a nonselective cholinesterase inhibitor.

When given alone, iso-OMPA inhibited mouse plasma butyrylcholinesterase activity by more than 99% at all doses tested (2, 4, 8, 12 mg/kg, s.c.), and no overt cholinergic signs (tremor, salivation) were observed at any dose. Although not determined directly, doses that produce this degree of plasma butyrylcholinesterase inhibition would be predicted to cause near-total inhibition of butyrylcholinesterase in peripheral ganglia and to provide greater than 50% inhibition of brain butyrylcholinesterase (Koelle et al., 1974). Iso-OMPA administered alone at doses from 2–4 mg/kg s.c. did not alter brain acetylcholine levels. A dose of 2 mg/kg s.c. was selected for further studies, as this dose was sufficient to provide complete, irreversible inhibition of plasma butyrylcholinesterase and minimized the possibility of nonselective inhibition of related serine proteases and esterases.

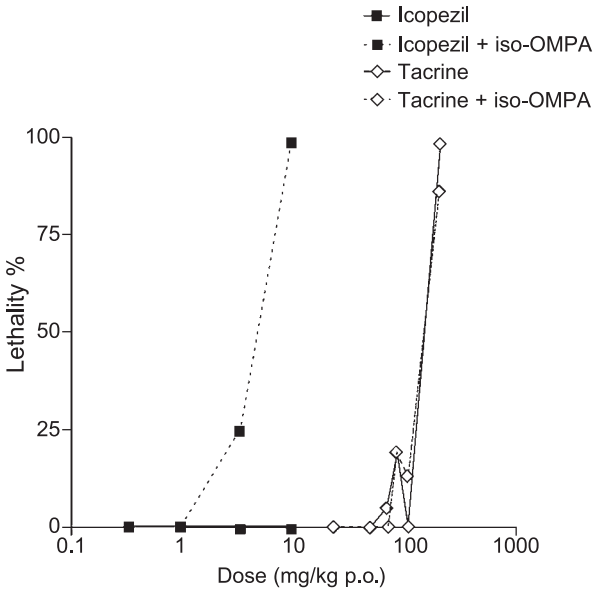

Fig. 5. The effects of the addition of iso-OMPA to icopezil- and tacrine-induced lethality in mice. Data were pooled from 2 experiments, in which n = 9 for each dose.

The addition of selective butyrylcholinesterase inhibition with iso-OMPA did not augment the increase in mouse brain acetylcholine induced by icopezil or tacrine (Fig. 3). The addition of iso-OMPA to mice treated with the highest dose of tacrine tested (56 mg/kg) yielded a significant reduction in the tacrine-induced increase in brain acetylcholine. Iso-OMPA had no effect on the centrally mediated tremors induced by either tacrine or icopezil (Fig. 4). In contrast, iso-OMPA potentiated the peripherally mediated salivation produced by icopezil, but had no effect on tacrine-induced salivation (Fig. 4). The acute lethality of icopezil was increased by the addition of iso-OMPA. Iso-OMPA had no detectable effect on the lethality of tacrine (Fig. 5).

4. Discussion

Clinical experience has shown that acetylcholinesterase inhibition is a viable therapeutic approach to the palliative treatment of Alzheimer's disease. Some of the most common untoward effects of such therapy are gastrointestinal complaints resulting from stimulation of peripheral autonomic cholinergic systems. We have examined whether some of the peripheral cholinergic effects could be due to nonselective inhibition of both acetylcholinesterase and butyrylcholinesterase. Furthermore, we designed experiments to determine whether addition of butyrylcholinesterase inhibition could enhance the central cholinergic effects of a selective acetylcholinesterase inhibitor. To achieve these goals, we examined the pharmacology of selective and nonselective cholinesterase inhibitors from four chemical classes. The benzylpiperidines (donepezil, icopezil) are highly selective for acetylcholinesterase, whereas the carbamates (physostigmine and analogues) and aminoacridines (tacrine) are nonselective, tending to be more potent at butyrylcholinesterase than acetylcholinesterase. The organophosphate, iso-OMPA, is a highly selective irreversible inhibitor of butyrylcholinesterase.

The benzylpiperidines are potent and selective acetylcholinesterase inhibitors in vitro. Indeed, inhibition of acetylcholinesterase is the only activity of donepezil or icopezil observed at concentrations expected to be clinically relevant. They are 2–3 orders of magnitude more potent at inhibiting acetylcholinesterase than at binding to acetylcholine receptors or biosynthetic enzymes. This high selectivity for acetylcholinesterase is evident from the molecular interactions of these agents with the cholinesterases. Acetylcholinesterase and butyrylcholinesterase display greater than 50% identity in their aligned primary sequences (Prody et al., 1987; Soreq et al., 1990). The three-dimensional structure of acetylcholinesterase in complex with donepezil has been reported (Kryger et al., 1999), and molecular modelling based on the crystal structure has illustrated the complementarity between this class of inhibitors and the narrow gorge of the protein leading to the catalytic site (Villalobos et al., 1994). In acetylcholinester-

ase, the active site gorge is rich in aromatic amino acid residues (Sussman et al., 1991). In butyrylcholinesterase, 6 of 14 aromatic residues that line this gorge have been replaced with aliphatic or basic residues (Harel et al., 1992). The aromatic character of donepezil and icopezil provides high complementarity with the active site gorge of acetylcholinesterase, particularly through interaction of residues tryptophan^{279} with the benzisoxazole and phenylalanine^{330} with the phenyl ring of icopezil (Villalobos et al., 1994). The presence of aliphatic residues in butyrylcholinesterase provides much less hydrophobic interaction with icopezil and is likely to contribute to the high degree of selectivity shown by this inhibitor.

In contrast to the benzylpiperidines, tacrine and heptylphysostigmine were potent butyrylcholinesterase inhibitors, and are thus relatively nonselective cholinesterase inhibitors. Modest interaction with muscarinic receptors (0.7–7 μM) was evident with tacrine, which may play a role in vivo given tacrine's relatively lower potency at acetylcholinesterase (0.11 μM). The in vitro experiments demonstrated that these compounds did not inhibit high affinity choline uptake and choline acetyltransferase, thus indicating that these drugs are unlikely to interfere with the synthesis of acetylcholine in vivo.

The cholinesterase inhibitors evaluated in the present study all produced increases in mouse brain acetylcholine following oral administration. All four inhibitors induced tremors in mice, an effect known to be associated with central cholinergic activation (Connor et al., 1966; Wishart and Herberg, 1979; Itoh, 1995; Stanford and Fowler, 1997). There were, however, substantial differences in the relative potency with which these cholinesterase inhibitors induced salivation, a response that is mediated predominantly by peripheral cholinergic activation (Sanchez and Meier, 1993; Domino and Corssen, 1967). The selective acetylcholinesterase inhibitors (icopezil and donepezil) induced tremors with a 3-fold greater potency than their induction of salivation. In contrast, the less selective compounds, tacrine and heptylphysostigmine, were equally potent at producing these centrally and peripherally mediated cholinergic effects.

The addition of iso-OMPA potentiated salivation and lethality when co-administered with icopezil, whereas no effect was observed on either of these parameters when iso-OMPA was co-administered with tacrine. By contrast, iso-OMPA had little effect on the increased brain acetylcholine levels or the induction of centrally mediated tremors produced by either tacrine or icopezil. This indicates that the combined inhibition of acetylcholinesterase and butyrylcholinesterase does not increase centrally mediated cholinergic effects above those produced by the selective inhibition of acetylcholinesterase alone. Conversely, peripherally mediated effects and lethality are increased by combined inhibition of acetylcholinesterase and butyrylcholinesterase compared with the selective inhibition of acetylcholinesterase alone.

The results of these experiments are very similar to those reported in earlier pre-clinical studies (Snape et al., 1999; Dronfield et al., 2000). In one study comparing the effects in rats of tacrine, donepezil, and the selective acetylcholinesterase inhibitor NXX-066 (3,4-Dihydro-1H-isoquinoline-2-carboxylic acid 1,3*a*,8-trimethyl-1,2,3,3*a*,8,8*a*-hexahydropyrrolo[2,3-*b*]indol-5-yl ester), tacrine was equipotent at inducing salivation and tremor (Snape et al., 1999). Donepezil was observed to be three times more potent at inducing tremor compared with salivation after i.p. administration. Similarly, oral dosing of donepezil induced high tremor scores, whereas only low levels of salivation were observed, even at the highest dose tested (Snape et al., 1999). In a study conducted in rats, donepezil, tacrine, rivastigmine, and metrifonate induced tremors in a dose-dependent manner (Dronfield et al., 2000). Tacrine and rivastigmine produced marked salivation at the higher doses tested, whereas donepezil and metrifonate were not observed to induce this peripheral effect (Dronfield et al., 2000). An additional study showed that the lethality of the irreversible acetylcholinesterase inhibitor soman could be potentiated by the co-administration of iso-OMPA (Gupta and Dettbarn, 1987). Thus, the peripheral actions and acute lethality of the cholinesterase inhibitors appear to be most strongly associated with butyrylcholinesterase inhibition under conditions where acetylcholinesterase is also inhibited.

The biological role of butyrylcholinesterase is unknown (Atack et al., 1987; Cooper, 1994), but it has been suggested to be a scavenger for toxins and to hydrolyze acetylcholine that escapes the action of acetylcholinesterase (Schwarz et al., 1995; Li et al., 2000). Butyrylcholinesterase is abundant in plasma and interstitial fluids of peripheral tissues, with moderate activity in the adult brain (Atack et al., 1987; Brimijoin and Hammond, 1988; Li et al., 2000). Acetylcholinesterase is widely distributed in neurons and axon terminals in brain and peripheral tissues, where it is responsible for terminating the action of acetylcholine (Atack et al., 1987; Francis et al., 1999). If butyrylcholinesterase is involved in cholinergic neurotransmission, its relative abundance in the periphery versus the brain suggests that it may play a much greater role in modulating peripheral effects versus central effects. The observations reported here are consistent with a role for butyrylcholinesterase in hydrolysis of acetylcholine that escapes degradation by acetylcholinesterase, since potentiation of salivation and lethality by butyrylcholinesterase inhibition was only observed when acetylcholinesterase was blocked by a selective inhibitor. Furthermore, the inhibition of butyrylcholinesterase alone induced by iso-OMPA did not cause tremors or salivation, indicating that inhibition of acetylcholinesterase is required for manifestation of cholinergic signs.

Humans lacking functional butyrylcholinesterase appear healthy (Primo-Parmo et al., 1996), which suggests that this enzyme is not essential for regulation of cholinergic function. No cases of total acetylcholinesterase deficiency in

humans have been reported (Li et al., 2000), although acetylcholinesterase knockout mice have been constructed (Xie et al., 2000). These mice show developmental delays and do not survive to adulthood without special care. Interestingly, acetylcholinesterase knockout mice are supersensitive to the toxic effects of organophosphates and the highly selective butyrylcholinesterase inhibitor bambuterol, indicating that extensive inhibition of both butyrylcholinesterase and acetylcholinesterase is lethal (Xie et al., 2000; Duysen et al., 2001).

It has been reported that in the brains of Alzheimer's disease patients, butyrylcholinesterase and acetylcholinesterase are found in and around the plaques and tangles (Weinstock, 1999). The biochemical properties of the butyrylcholinesterase from Alzheimer's disease brain appear to differ from those in control brain, showing a reduced optimum pH and altered sensitivity to inhibitors (Geula and Mesulam, 1989; Schatz et al., 1990). These and other findings suggest that agents which inhibit both butyrylcholinesterase and acetylcholinesterase may have advantages over selective acetylcholinesterase inhibitors (Weinstock, 1999). A comparison of published clinical trial data and the findings from a randomized comparative trial of the selective acetylcholinesterase inhibitor donepezil and the nonselective inhibitor rivastigmine suggest similar efficacy on measures of cognition (Wilkinson et al., 2002). These agents have in common the mechanism of acetylcholinesterase inhibition, which has been shown to be positively correlated with changes in cognition both in laboratory animals and in Alzheimer's disease patients (Rogers et al., 1998; Woodruff-Pak et al., 2001; Imbimbo, 2001). However, it is not known if either inhibitor blocks the altered butyrylcholinesterase activity reportedly present in plaques.

In conclusion, the pre-clinical data reported here indicate that the more selective acetylcholinesterase inhibitors (benzylpiperidines) produce fewer peripheral cholinergic signs than nonselective cholinesterase inhibitors. This is consistent with findings of clinical trials in Alzheimer's disease patients, demonstrating that the combined inhibition of acetylcholinesterase and butyrylcholinesterase is unlikely to be associated with superior efficacy, but rather with a greater incidence of cholinergic adverse events and reduced therapeutic index.

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