Inhibition of acetylcholinesterase from electric eel by (−)- and (+)-physostigmine and related compounds

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Received 17 April 1986

Unnatural (+)-physostigmine (**2**) inhibited acetylcholinesterase (AChE) from electric eel considerably less than natural (−)-physostigmine (**1**), but **2** may because of its possible lower toxicity still be an interesting anticholinesterase agent. (−)-Eseroline (**3**), a major metabolite of (−)-physostigmine (**1**) and a potent narcotic analgetic, and its unnatural (+)-antipode (**4**), were both poor inhibitors of the enzyme. (−)-Dihydrosecophysostigmine (**5**), a ring-open analog of (−)-physostigmine was less, but (−)-*N*-methylphysostigmine (**6**) much more potent than the natural alkaloid. The availability of compounds related to (−)- and (+)-physostigmine by improved chemical synthesis suggests that further structural variation may well lead to other biologically interesting AChE inhibitors.

Physostigmine (Eserine) Eseroline Cholinesterase

1. INTRODUCTION

Regulation of acetylcholine turnover and level in neurons and synaptic junctions may play an important role in a number of neurologic disorders, particularly Alzheimer's disease, myasthenia gravis, and anticholinesterase poisoning. The alkaloid (−)-physostigmine (**1**), also called (−)-eserine, is known for its antiacetylcholinesterase properties, and the way it interferes with the enzyme is rather well understood [1]. Our aim to reinvestigate compounds related to this alkaloid, and to perform a qualitative structure-activity study, was prompted by a much improved synthesis of (±)-*N*1-noreseroline *O*-methyl ether, and its optical resolution using urea derivatives [2,3], giving easy access to a wide variety of natural and unnatural analogs of physostigmine. We wanted to confirm that unnatural (+)-physostigmine (**2**), found to be a much less potent inhibitor of erythrocyte acetylcholinesterase (AChE) [4], was also less potent in our assay. We furthermore included the ring-open analog **5** of (−)-physostigmine, (−)-*N*-methylphysostigmine (**6**), as well as the two phenols (−)- and (+)-eseroline (**3** and **4**, respectively) hoping that the already established qualitative profile of structure and anti-cholinesterase activity of compounds related to physostigmine [4] could be ascertained.

2. MATERIALS AND METHODS

(−)-Physostigmine salicylate (**1**) was purchased from Fluka (Hauppauge, NY). The salicylate of (+)-physostigmine (**2**) was obtained by reaction of (+)-eseroline (**4**) prepared by total synthesis [2–4], with methyl isocyanate, and treatment of the free base with salicylic acid in ether. (−)-Eseroline (**3**) was prepared from **1** as in [5], and converted into its sulfate salt with sulfuric acid in acetone. (−)-Dihydrosecophysostigmine (**5**), obtained earlier from **1** by reduction with zinc in HCl [6], was prepared here by reduction with sodium

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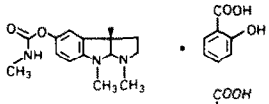
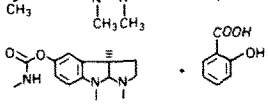
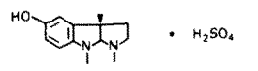
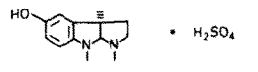
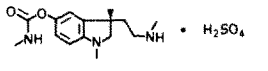
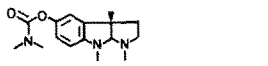
Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

Table 1

No.	Name	Molecular Comp.	Formula	M.W.	M.p.	$[\alpha]_D^{RT}$	Remarks
①	(−)-Physostigmine-salicylate (natural) ○	$C_{22}H_{27}N_3O_5$		413.47	182-184°	-70.5° (c=0.8 in $CHCl_3$)	
②	(+)-Physostigmine-salicylate (unnatural) ●	$C_{22}H_{27}N_3O_5$		413.47	180-182°	+67.3° (c=0.6 in $CHCl_3$)	
③	(−)-Eseroline-sulfate △	$C_{13}H_{20}N_2O_5S$		316.38	210-212°	-118.4° (c=1.1 in MeOH)	hygros.
④	(+)-Eseroline-sulfate ▲	$C_{13}H_{20}N_2O_5S$		316.38	200-206°	+ 116.0° (c=1.1 in MeOH)	hygros.
⑤	(−)-Dihydro-physostigmine-sulfate □	$C_{15}H_{25}N_3O_6S$		375.44	165-170°	+13.1° (c=1.2 in MeOH)	extremely hygros.
⑥	(−)-N-Methyl-physostigmine ■	$C_{16}H_{23}N_3O_2$		289.38	oil	-64.2° (c=1.4 in $CHCl_3$)	

cyanoborohydride in aqueous methanol, in the presence of a catalytic amount of HCl, and converted to its sulfate salt in acetone. (−)-*N*-Methylphysostigmine (**6**) was prepared from **3** by reaction with dimethylcarbamoyl chloride in pyridine. Physical data obtained for the compounds described are listed in table 1.

Affinity-purified electric eel AChE was prepared in-house according to Yamamura et al. [7] for guinea pig brain. Acetylthiocholine and 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) were purchased from Sigma (St. Louis, MO). Buffers and other chemicals used were analytical reagent grade.

AChE activity was determined colorimetrically using acetylthiocholine as the substrate by a modified micro Ellman AChE assay [8], as described by Brogdon and Dickinson [9]. All reactions were carried out in 100 mM potassium phosphate buffer (pH 7.4) at room temperature. In a typical assay 40 μl buffer, 30 μl enzyme preparation, and 30 μl test compound solution were mixed and kept for 15 min. Enzymatic reaction was initiated by adding to this mixture 200 μl solution containing acetylthiocholine and DTNB. The time course of acetylthiocholine hydrolysis by AChE was determined by monitoring the increase of yellow color produced from the reaction of thiocholine with dithiobisnitrobenzoate ion at a wavelength of 414 nm.

3. RESULTS AND DISCUSSION

We confirm that inhibition of AChE from electric eel by natural (−)-physostigmine (**1**), as shown in table 2, is enantioselective, and that unnatural (+)-physostigmine (**2**) is about 125-times less potent in this assay. (−)-Eseroline (**3**), a major metabolite of (−)-physostigmine and a potent nar-

Table 2

Relative anticholinesterase potencies of physostigmine derivatives

Compound	IC_{50} (M)
(−)-*N*-Methylphysostigmine	1.8×10^{-10}
(−)-Physostigmine salicylate	4.0×10^{-9}
(−)-Dihydrosecophysostigmine sulfate	1.0×10^{-7}
(+)-Physostigmine salicylate	5.0×10^{-7}
(−)-Eseroline sulfate	1.5×10^{-6}
(+)-Eseroline sulfate	7.0×10^{-6}

IC_{50} (test compound concentration required to inhibit 50% eel AChE in vitro) values were obtained from plots of enzyme activity vs concentration of compounds shown in fig.1

cotic analgetic [10,11], and its optical isomer (+)-eseroline (**4**) [11] were, as shown in table 2, several hundred times less potent than the natural alkaloid **1**. The ring-open dihydroseco analog **5** is about 25-times less potent than (−)-physostigmine from which it is derived, suggesting that optimal potency may possibly require a tricyclic, rather than a bicyclic template [12]. Whether the remarkably high potency of (−)-*N*-methylphysostigmine (**6**), in agreement with findings made in the pyridostigmine family of compounds [13], is due to steric effects, better lipophilicity, or other factors, remains to be determined, but clearly suggests that more potent compounds can probably be discovered in the physostigmine family.

Whether (+)-physostigmine (**2**), because of its possibly lower toxicity but still respectable anticholinesterase activity, is of use in neurologic disorders or anticholinesterase poisoning is being investigated.

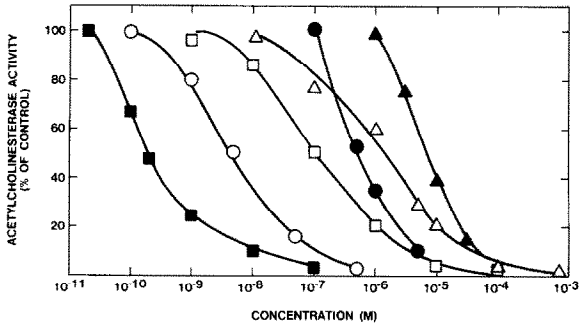


Fig.1. Effects of different test compounds on eel AChE activity. AChE was assayed colorimetrically as described in section 2. Amount of enzyme activity at each concentration of the test compounds shown was expressed as percent of control activity without any compound included in the assay. ○, (−)-physostigmine salicylate; ●, (+)-physostigmine salicylate; △, (−)-eseroline sulfate; ▲, (+)-eseroline sulfate; □, (−)-dihydroseco-physostigmine sulfate; ■, (−)-*N*-methylphysostigmine. Each point is the mean of 4–8 assays. The assay results were highly reproducible with a variation of approx. 5% or less from the mean

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