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Effect of cholinesterase inhibition *in vitro* by huperzine analogs

TANG Xi-Can, XU Hong[1], FENG Jie, ZHOU Tian-Xi[2], LIU Jia-Sen[2]
(*Department of Pharmacology, [2]Department of Phytochemistry, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031, China*)

ABSTRACT Huperzine (Hup) A and B were first discovered and studied by Chinese. Fourteen analogs of Hup were tested for their anticholinesterase (anti-ChE) activity by colorimetric method using rat erythrocyte membrane and serum as enzyme sources. Hup-A showed the highest anti-AChE potency. The anti-BuChE activity of (—)dihydro and (—) tetrahydro analogs were potent than those of Hup-A. Their anti-AChE activities were comparable to those of physostigmine and galanthamine. Dihydro analog inhibition was of the mixed competitive type with a K_i value of 0.12 μmol·L^{-1}. Tetrahydro analog inhibition was of the competitive type with a K_i value of 0.56 μmol·L^{-1}. They were different from isoflurophate to bond to AChE in a reversible manner.

KEY WORDS huperzine; physostigmine; galanthamine; isoflurophate; erythrocyte membrane; cholinesterase inhibitors; structure-activity relationship

Huperzine A (Hup-A) and huperzine B (Hup-B), 2 new licopodium alkaloids isolated from Chinese herb *Huperzia serrata* (Thumb) Trev[1], were first discovered and studied by Chinese. They were potent and selective acetylcholinesterase (AChE) inhibitors[2,3] with better therapeutic indexes than that of physostigmine[4].

Hup-A and B were effective in a variety of behavior tests for appraising animal learning and memory function[5]. Clinical trial showed that Hup-A was a promising therapeutic agent in the treatment of senile memory disorders due to its longer duration of action[6,7] with minimal side effects. These findings have captured the interest of many researchers because it is possible to be a key structure in the treatment of Alzheimer's disease. In the efforts of searching for more potent analog of Hup-A, none of the analogs has yet achieved the potency of parent compound[8—11].

Herein we report the anti-ChE activity of 14 analogs prepared from nature Hup to explore the structure-activity relationship.

MATERIALS AND METHODS

Rat erythrocyte membrane and serum were used as AChE and butyrylcholinesterase (BuChE) enzyme sources. Erythrocyte membrane prepared as described previously[12] was stored at — 30 °C. Acetylthiocholine iodide, 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB), physostigmine salicylate (Phys), and isoflurophate purchased from Fluka Chemie. Butyrylthiocholine iodide was a product of Koch-Light Lab, UK. Sodium lauryl sulphate (SDS) was bought from BDH, UK. Galanthamine hydrobromide (Gal) was a product of Shanghai Hai Pu Pharmaceutical Works. Phosphate buffer and Tris-HCl buffer were prepared using domestic products of AR. Natural Hup-A, Hup-B and their semi-synthetic analogs were prepared in our laboratory.

For assay of anti-ChE activity of analogs, a reaction mixture of 4 ml containing acetylthiocholine iodide 0.3 mmol·L^{-1} or butyrylthiocholine iodide 0.4 mmol·L^{-1}, phosphate buffer (0.1 mol·L^{-1}, pH 7.4) 1 ml, tested analog 0.1—0.3 ml, and enzyme 0.1—0.2 ml was incubated at 37 °C for 8 min. The reaction

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[1] Now in *Department of Biology, Suzhou Railway Teacher's College, Suzhou 215009, China.*

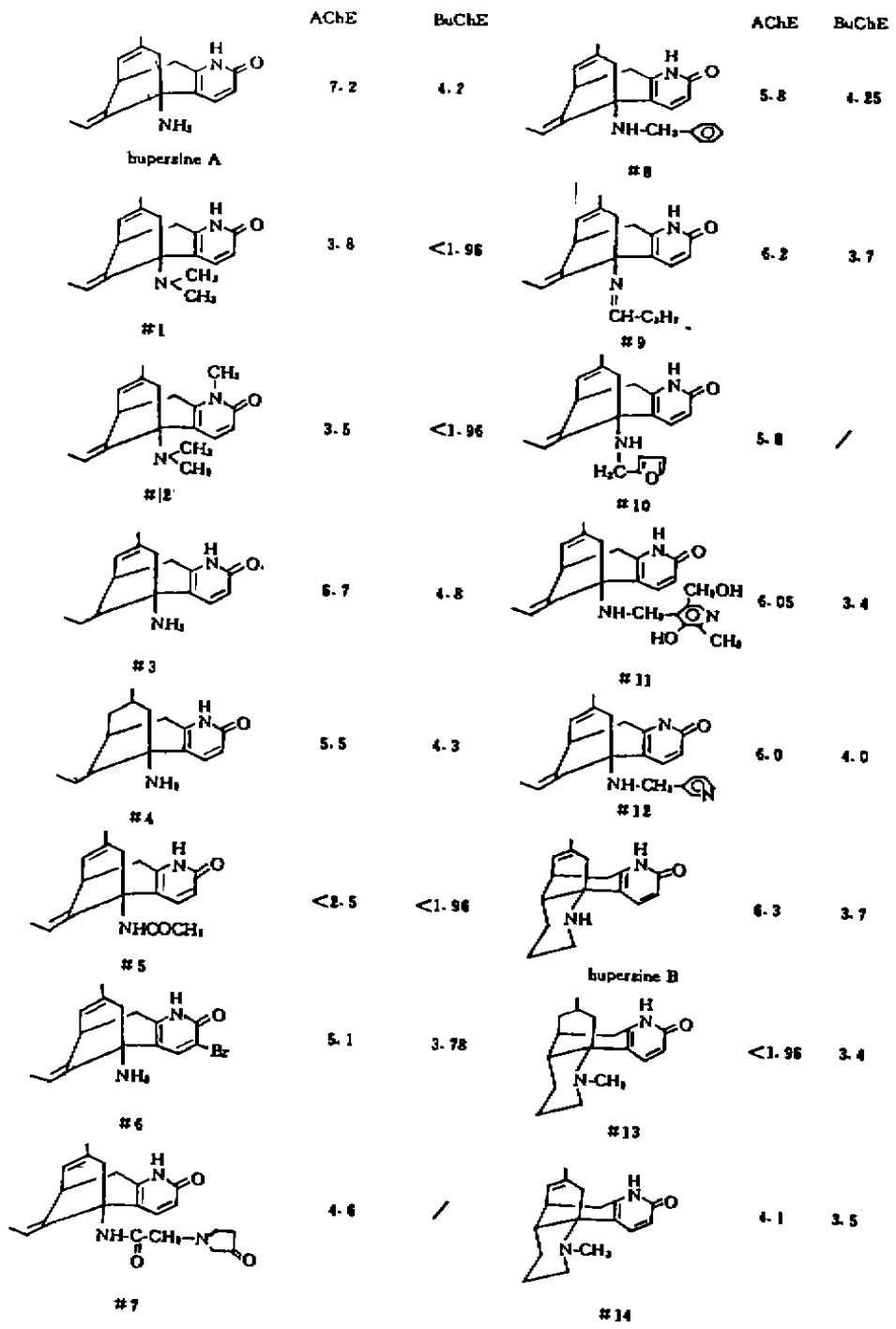

Fig 1. pI_{50} **of anti-ChE activities by Hup analogs on rat erythrocyte membrane (AChE) and rat serum over a concentration range from 1 mmol·L^{-1} to 10 mmol·L^{-1}.**

was terminated by adding 1 ml of 3 % SDS, then 1 ml of 0.2 % DTNB was added to produce the yellow anion of 5-thio-2-nitro-benzoic acid. The rate of color production was measured spectrophotometerically at 440 nm[12]. The mechanism of inhibition and K_i values for analogs were determined by using the method of Lineweaver and Burke[12].

RESULTS AND DISCUSSIONS

Inhibitions of ChE activity on 14 semi-synthesized analogs were tested over a concentration range from 1 nmol·L^{-1} to 10 mmol·L^{-1}. The pI_{50} (negative logarithm of molar concentration causing 50 % inhibition of ChE) for AChE and BuChE inhibition revealed that Hup-A showed the highest anti-AChE potency on rat erythrocyte membrane *in vitro*. Removal of exocyclic double bond (analog 3) or both exocyclic and endocyclic double bonds (analog 4) led to a diminution of anti-AChE activity, whereas the anti-BuChE activity exhibited potent than that of Hup-A. Modification in the vicinity of amino group resulted in less activity (Fig 1).

Among the analogs tested, analog 3 exhibited comparable anti-AChE activity to Phys. The anti-AChE activity of analog 4 close to Gal. (Fig 2)

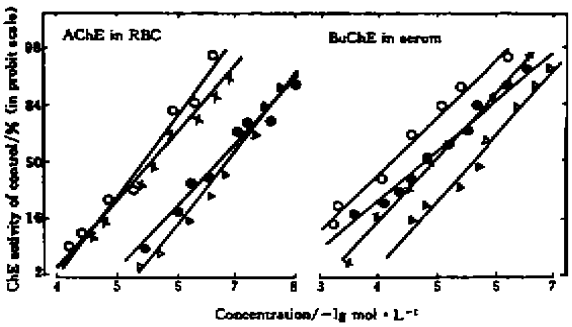

Fig 2. Anti-ChE activities of analog 3 (●), physostigmine (△), analog 4 (□), and galanthamine (×).

The AChE activity did not exhibit progressive decrease with the prolongation of incubation with analogs 3 and 4. Their inhibitory manners were different from that of isoflurophate. (Fig 3)

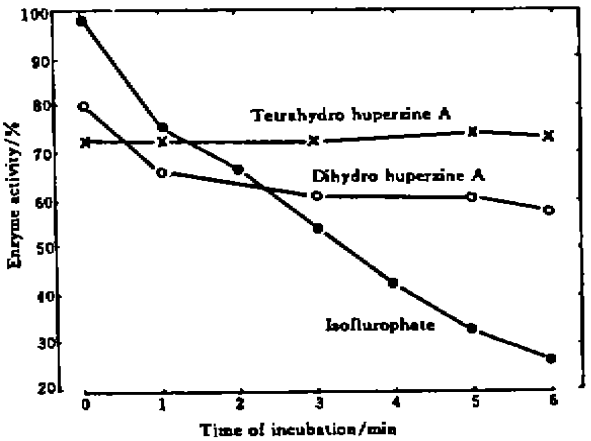

Fig 3. Rat erythrocyte membrane AChE activity after incubation with Hup-A analogs and isoflurophate.

Analog 3 or 4 0.1 ml with AChE 0.4 ml was incubated at 37 ℃ for 3 min. The reaction mixture was washed 5 times with 3 ml of Tris-buffer (50 mmol·L^{-1}, pH 7.4) and spun at 18 000 × g. The erythrocyte membrane AChE preincubated with analog 3 or 4 recoved their activities to 101 % or 98 % of the control, respectively. It indicates that analog 3 or 4 combines to AChE in a reversible way.

Lineweaver-Burke plot for analog 3 indicated a pattern of inhibition of AChE of the mixed competitive type as the intersection of the lines occured in the second quadrant, whereas analog 4 belonged to a competitive type as the line intersected on the Y-axis. For analog 3 the K_i and K_iS values were 0.16 and 1.03 μmol·L^{-1}, respectively. The K_i value of analog 4 was 0.56 μmol·L^{-1}. Hup-A (23 nmol·L^{-1}) was 7 and 24 times respectively as potent as those of analog 3 and analog 4. (Fig 4).

Based on the above-mentioned results, it indicated that structural requirements for high

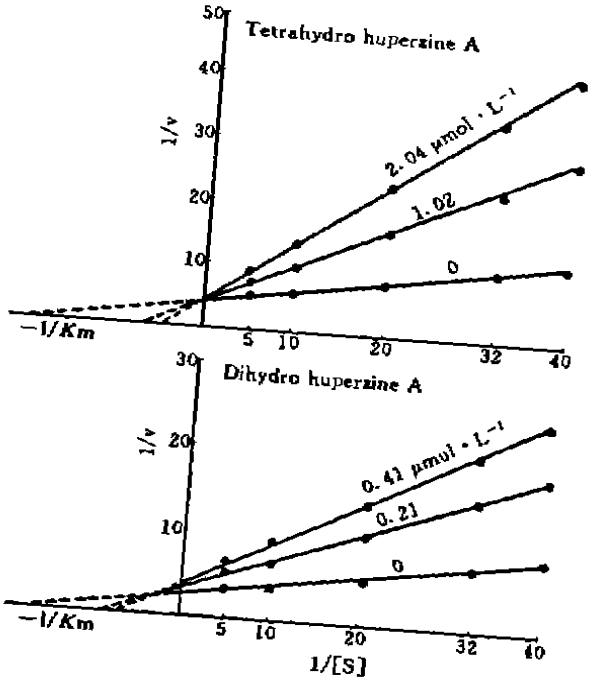

Fig 4. Double-reciprocal plots of rat erythrocyte membrane AChE after analog 3 and analog 4.

anti-AChE activity in the molecular of Hup-A appear to include a properly positioned amino group, a double bond in the bridge and an exocyclic E double bond.

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石杉碱类似物在体外对胆碱酯酶的抑制作用

唐希灿，徐 泓[1]，冯 洁，周天锡[2]，刘加森
（中国科学院上海药物研究所药理室，[2]植物化学室，上海200031，中国）

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摘要 用比色法测试表明，14个半合成类似物的抗 AChE 作用均弱于 Hup-A. 左旋二氢及四氢类似物的抗 BuChE 作用稍强于 Hup-A. 前者属混合型抑制剂，K_i 值为0.12 μmol·L⁻¹，后者属竞争型抑制剂，K_i 值为0.56 μmol·L⁻¹，二者不同于异氟磷，与 AChE 为可逆性结合.

关键词 石杉碱；毒扁豆碱；加兰他敏；异氟磷；红细胞膜；胆碱酯酶抑制剂；构效关系