



JUN 30 2000
7

Memorandum

Date
From Acting Division Director, Division of Standards and Labeling Regulations, Office of
Nutritional Products, Labeling and Dietary Supplements, HFS-820
Subject 75-Dau Premarket Notification for New Dietary Ingredients
To Dockets Management Branch, HFA-305

| | |
|-------------------------|--------------------|
| New Dietary Ingredient: | <i>Huperzine A</i> |
| Firm: | NOW |
| Date Received by FDA | May 23, 2000 |
| 90-Day Date: | August 20, 2000 |

In accordance with the requirements of section 413(a) of the Federal Food, Drug, and
Cosmetic Act, The attached 75-day premarket notification for the aftermentioned
new dietary ingredient should be placed on public display in docket number

95S-0316 after August 20, 2000

Felicia B. Satchell
Felicia B. Satchell

95S-0316

RPT 75



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

JUN 30 2000

Food and Drug Administration
Washington, DC 20204

Al Powers
Vice President
NOW Foods
395 S. Glenn Ellyn Road
Bloomington, Illinois 60108

Dear Mr. Powers:

This is to notify you that your submission pursuant to section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act dated April 26, 2000, concerning the marketing of a substance that you assert is a new dietary ingredient (i.e., Huperzine A) was received by the Food and Drug Administration on May 23, 2000. Your submission will be kept confidential for 90 days from the date of receipt, and after August 20, 2000, your submission will be placed on public display at Dockets Management Branch (Docket No. 95S-0316). Commercial and confidential information in the notification will not be made available to the public.

Please contact us if you have any questions concerning this matter.

Sincerely yours,

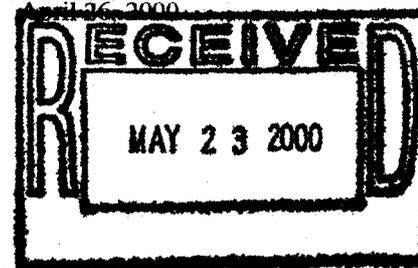
Felicia B. Satchell
(Acting) Division Director
Division of Standards
and Labeling Regulations
Office of Nutritional Products, Labeling
and Dietary Supplements



The Future in Natural Foods

Office of Special Nutritionals (HFS-450)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street SW
Washington, DC 20204

70848



RE: Notification of a New Dietary Ingredient

Dear Sir/Madam,

In compliance with the Dietary Supplement Health and Education Act of 1994, NOW Foods hereby makes its Notification of a New Dietary Ingredient, Huperzine A. Enclosed are two (2) copies of this Notification.

1. Name and Address of the Manufacturer
**NOW Foods
395 S. Glen Ellyn Rd.
Bloomington, IL 60108 USA**
2. Name of the new Dietary Ingredient
Huperzine A
3. Description of the Dietary Supplement containing the new Dietary Ingredient
Dietary supplement Brain Elevate contains Huperzine A, an alkaloid compound extracted from the herb *Huperzia serrata* present in a vegetable capsule form.

(a) The level of the new dietary ingredient is:
25mcg per vegetable capsule

(b) The conditions of use suggested on the label are:
**Suggested use: As a dietary supplement, take 1 Vcap™ 1 to 2 times daily.
Do not exceed dosage without the advice of a physician.**

Enclosed please find documentation that establishes this dietary ingredient, Huperzine A, when used under the conditions suggested on the label, will reasonably be expected to be safe. This documentation includes a Certificate of Analysis, toxicity information, review articles and efficacy studies.

An original and two copies of this notice are being filed. Pursuant to 21 CFR 190.6(c), please confirm your receipt of this notice.

Thank you for your time and attention to this matter. If you have any questions or comments, please do not hesitate to contact the undersigned.

Sincerely,
NOW FOODS

Al Powers
Vice President

Enclosure

CERTIFICATES OF ANALYSIS

Quality Assurance**Product Name: Huperzine A Powder-- 0.1% trituration on CaCO₃**

Description: Huperzine A [(-)-HupA] is a natural compound isolated in an extract of the club moss, *Huperzia serrata*, (also known as *Lycopodium serratum* Thumb) which grows at high elevations and in cold climates. It is a Chinese folk medicine, called *Qian Ceng Ta* and has been used for centuries traditionally to treat fever and inflammation, and recently to improve memory, focus and concentration and helps alleviate memory problems among the elderly. Reports from China, where it is used as a treatment, indicate that Huperzine A is safe and effective. It has other properties such as protecting nerve cells from toxic substances including nerve gas poisons, and from damage produced by strokes and epilepsy. *Huperzia* extract contains a wide variety of alkaloids, including lycodoline, lycoclavine, and serratinine, as well as the huperzines. Based on the laboratory studies some researchers believe that Huperzine A- a *Lycopodium* alkaloid may be more effective as a treatment of Alzheimer's disease. Scientific research has shown Huperzine A to be potent, selective and reversible inhibitor of AChE (Acetylcholinesterase) – the enzyme that breaks down acetylcholine- a neurotransmitter, with longer duration of action and minimal side effects. In other words Huperzine A has a superior safety and efficacy profile compared to other cholinesterase inhibitors. The chemical name of HupA is: (5R, 9R, 11E)-5-amino-11-ethylidene-5, 6, 9, 10-tetrahydro-7-methyl-5, 9-methanocycloocteno[b]pyridin-2 (1H)-one. Molecular weight is 242.32, (C₁₅H₁₈N₂O) (Merck Index # 4791). Natural Huperzine A is 3 times more potent than the synthetic forms, which is racemic mixture with 1:1 ratio of (-)-HupA, the natural existing compound, and (+)-HupA (38 fold less potent by itself). Other components like Huperzine B, found in the herb has been suggested to be beneficial as well. NOW Huperzine A is standardized to 0.1% trituration on Calcium Carbonate.

Vendor(s): Wilke Resources**Vendor's Code:** N/A**Quantity:** pending per drum/case**Color/Appearance:** A White or slight yellow crystalline free flowing needle-like crystalline powder.**Taste/Odor:** slight bitter taste, no odor**Mesh Size:** NLT 100% through # 80 US Standard sieve, NLT 95% through #100 US Standard sieve.**Powder Density:** *Tap (Pack):* 0.779 – 0.861g/ml*Untap (Loose/Bulk):* 0.437 – 0.483g/ml**Assay:** 98.0%- 102.0% purified HupA from extract (Standardized to NLT 0.1% trituration on Calcium Carbonate)**Specific Gravity (@ 25°C, w/w):** N/A**Optical rotation ([α]_D @24.5°C):** -150.4° (c=0.498 in MeOH)**pH:** pending**UV Absorbance (λ_{max}):** 231nm, 313nm (ethanol)**Refractive Index (@ 20°C, n_D):** N/A**Melting point:** 227 – 231°C (also reported at 214-215°C)**Flash point (Open/Closed cup):** N/A**Infrared Adsorption:** ___% at ___ (pending)**Freezing point (10% aq. Soln., w/w):** N/A (solid)**Boiling point (@ 760mm Hg):** N/A (solid)**Solubility:** *Water:* pending*Alcohol:* pending*Acetone:* pending*Other:* pending

Item # 52400

Product Class: 995

Solvent Used: *For Extraction:* pending
Solvent Residue: pending
Sterilization Method: N/Av

For Wash: N/A
Sterilization Residue: pending
Processing: *Bleaching:* N/A

Bromating: N/A

Ash (Residue on Ignition): pending

Moisture (Loss on drying): NMT 5.0%

Expiration Date (from time of Mfg.)/ Shelf Life: (pending) Chemical stability of HupA is excellent. It is resistant to structural changes at different temperatures when placed in acidic or alkaline solutions, thus indicating that HupA will persist longer in the body, and that tablets or capsules will have a longer shelf life. The terminal half-life of Hup A is 4.8 hours. *Test Method for Shelf Life:* pending

Storage: Store in a cool, dry, and dark environment in a tightly sealed original container.

Temperature for Storage: pending **Moisture Free:** Y (very hygroscopic material) **Low Oxygen:** pending

Maximum Inventory Storage (Weeks):

Room Temperature: Pending

Refrigeration: Pending

Freezer: Pending

Retail Storage Temperature: Temp. pending

Packaging – Special Requirements (Inserts): 4 x 4oz Desiccants for bulk. Incompatible with strong oxidizing agents.

Specifications- Ingredients by Reference Number, Weight per level teaspoon:

| Ref. | Weight/ Tspn | Ingredients |
|-------|------------------|--|
| 52400 | 2250-2300mg/tspn | Huperzine A ^o (0.1% trituration on CaCO ₃) powder (Wilke Resources) |

Impurities (maximum permitted levels):

Chemical By-Products/ Deterioration Products: Not Known **Pesticides:** None **Alkaloid Impurities:** Absent

Microbiological / Heavy Metals: Standard Plate Count: < 100,000/g; Yeast and Molds: < 2,000/g; Coliform: < 300/g; E. coli & Salmonella: Negative

Heavy Metals: HM as Lead (Pb): <10ppm; Lead: <5ppm; Arsenic/ Cadmium/ Mercury/Aluminum: <1ppm (each)

All bulk powder ingredients are GMO free (Not genetically engineered)– if available, Non-High Energy Processed (a.k.a. irradiation, Microwaving), contain no artificial flavors or colors, and are preservative free. Only solvents or Manufacturing aids approved by NOW Foods may be used in its production.

Toxicity: LD₅₀ in rats (ml/kg): 4.6 orally. Hup A is safe because it would take more than 20 times the therapeutic dose (0.2mg/kg, oral) to reach the LD₅₀.

NOW QC Product Tests: BT, ME, HM, AS, RI, OR, SO, CO, PH, MP, PT

Assay Method (active ingredient (s)): HPLC for Huperzine A

Approved Labs: American Analytical / Industrial / NOW Foods/ Plant Bioactives

Distribution Rights (per Legal Counsel): No restrictions (pending)

Date Issued: 10-14-99

Supersedes: None

Approved: Jim Roza *JR*
Nilesh Patel *NP*

^o Pregnant women, and people with hypertension and pulmonary problems should not take it.

A : TJ SUNLIGHT INTERNATIONAL

TEL NO. : 0086 22 24463071

SEP. 10 1999 12:12PM P. 2

WENLING PHARMACEUTICAL FACTORY CERTIFICATE OF ANALYSIS



| | | | |
|-------------|----------------------|--------------|-----------------------|
| Sample name | Hyperzine A | Quantity | 1g |
| Packing | Plastic bottle | Batch size | 250g |
| Deliverer | Plant-extra-workshop | Manufacturer | Wenling pharm factory |
| Batch No | 990710 | Rec date | 99.07.10 |
| Criterion | WS-127(X107)-94 | Rep.date | 99.07.13 |

RESULT

1. Characteristics:

A white needle-like crystalline powder, odorless, hygroscopic.

2. Melting point:

228.0-229.0°C (should be 227-231°C)

3. Identification:

(1) λ max: 231nm, 313nm

(2),(3) positive

4. Loss on drying:

2.7% (not more than 5.0%)

5. Related substances:

Alkaloid impurities I ? in accord

Alkaloid impurities II ? in accord

Other impurities in accord

6. Assay:

99.3% (should be 98.0-102.0%)

Conclusion:

This batch of product is conformity with the above criterion.

UV absorbance

Insp. Manager: Chen Jianlong

Tester: Chen Yaping

HUPERZINE A

TYPICAL CERTIFICATE OF ANALYSIS

| | | | |
|-------------|-----------------|----------------|----------|
| Sample Name | Huperzine A | Quantity | 0.3 g |
| Packing | Plastic Bottle | Batch Size | 350 g |
| Batch No. | 9711-01 | Receiving Date | 11/3/97 |
| Criteria | WS-127(X107)-94 | Reporting Date | 11/10/97 |

RESULTS

| Analysis | Specification | Actual |
|--------------------|--|------------------|
| 1. Characteristics | White needle-like crystalline powder, odorless & hydroscopic | |
| 2. Melting Point | 227 to 231 °C | 228.5 - 229.5 °C |
| 3. Identification | (1) λ_{max} : 231 nm, 313 nm (2), (3) positive | Conforms |
| 4. Loss on Drying | 5.0% Max. | 1.21% |
| 5. Assay | 98.0 - 102.0% | 101.3% |

Conclusion: This batch of product is in conformity with the above criterion.

Insp. Manager: _____ Checker: _____ Tester: _____

[Note: The Certificate of Analysis that accompanies the actual shipment of product will have the seal of the pharmaceutical manufacturer on it]

TOXICITY INFORMATION

#15 Dup.

MEMORY MOSS

Huperzine A is a natural compound isolated from the club moss, *Huperzia serrata*. It is a Chinese folk medicine, called "qian ceng ta" and has been used for centuries to improve memory, focus and concentration and to help alleviate memory problems among the elderly. Reports from China, where an estimated 100,000 people have been treated, indicate that Huperzine A is safe and effective. It has other properties such as protecting nerve cells from toxic substances including nerve gas poisons, and from damage produced by strokes and epilepsy. It is also used to treat persons afflicted with Alzheimer's disease and myasthenia gravis. Based on laboratory studies some researchers believe that Huperzine A may be more effective than drugs presently available for the treatment of Alzheimer's disease.

ABOUT THE AUTHORS

*Debasis Bagchi, Ph.D., F.A.C.N., received his doctorate in medicinal chemistry from the Indian Institute of Chemical Biology in India and is an Associate Professor at Creighton University School of Pharmacy and Allied Health Professions, Omaha, NE. His research interests include mechanisms of toxicity induced by heavy metals and environmental pollutants, and the design, synthesis and evaluation of novel antioxidants/chemoprotectants. Dr. Bagchi has over 81 publications in peer-reviewed scientific journals. He is a Fellow of the American College of Nutrition, and a member of the New York Academy of Sciences, the Society of Toxicology and the American Association for the College of Pharmacists.

*Jean Barilla, M.S., is a medical writer, editor and author of books on health and nutrition. She has been published in scientific journals, medical-legal textbooks, magazines and newspapers. Jean lectures to groups and is a frequent radio guest. She edits a journal for physicians and other health professionals who use nutritional therapies, and has also been a staff biologist for the Food and Drug Administration, a lecturer at the City University of New York and author of medical education materials for physicians and other health professionals.

Huperzine A is intended solely for informational and educational purposes, and not as medical advice. Please consult a medical or healthcare professional if you have questions about your health.

HUPERZINE A: BOOST YOUR BRAIN POWER

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INTRODUCTION

The aging process, physical and mental stress, and environmental toxins induce deleterious effects in the human brain. Initial symptoms can include short-term memory loss, lack of focus and reduced concentration. More seriously, they can lead to degenerative diseases of the brain including long-term memory loss and Alzheimer's dementia, the fourth leading cause of death in the United States.

The harmful effects of environmental toxins alone on the nervous system or brain are a major threat to the human race. Many people will recall the case of Minamata disease in Japan in the 1950s and 1960s and in Iraq in 1971-72. Those who ate fish and shellfish contaminated by methylmercury developed damage of the brain, spinal cord and peripheral nerves (those coming from the brain and spinal cord). Symptoms of the disease included disruption of visual and sensory nerve function, muscle uncoordination (ataxia) and impairment of hearing, speech and gait. Chronic poisoning from environmental toxins can also cause general weakness of the extremities, memory loss, multi-infarct dementia (resulting from small strokes) and various forms of memory impairment including Alzheimer's dementia. Dementia is a loss of intellectual function (thinking, remembering, reasoning) so severe that it interferes with an individual's daily functioning and eventually results in death. While the causes of Alzheimer's disease are not known and are currently undergoing intense scientific investigation, suspected causes include defective genes or a genetic predisposition, abnormal protein build-up in the brain (this will be explained later) and environmental toxins.

While the liver and other organs can regenerate and form

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new cells after a toxic insult, brain or nerve cells which die as a result of irreversible damage are not replaced. Alzheimer's disease is a degenerative disease. There is a progressive loss of nerve cells (neurons), and the loss is selective, meaning that it affects one or more groups of neurons while leaving others intact. Also, the disease arises without any clear "inciting event" in a patient having no known (previous) neurologic deficits. Although most cases are sporadic, at least 5 to 10 percent of cases have a genetic (hereditary) component. For Alzheimer's, the destruction occurs in the cerebral cortex, which is the outer tissue of the brain—the "gray matter." This tissue is made up of nerve fibers involved in movement, sensation, vision, hearing and other higher brain functions. The main symptom of degeneration of these nerves is dementia, i.e., progressive loss of cognitive function independent of the state of attention. Dementia is not part of normal aging and always represents a disease process. The best that can be done for Alzheimer's patients is to help them use their remaining healthy brain tissue fully, and protect their brains from the ravages of the disease. One characteristic that all degenerative brain diseases, including Alzheimer's, share is that oxidative stress increases the rate at which the disease progresses.¹

Oxidative stress results from free radical damage. Free radicals are destructive molecules that are produced in the body as a result of exposure to environmental toxins or occur as a result of normal body metabolism. Free radicals damage cells in every part of the body. Brain cells, however, are particularly susceptible to oxidative stress for two reasons. First, nerve cells do not divide, so no new cells are made. The brain thus has no means of escaping damage through cellular replenishment. Second, neurons possess low levels of antioxidant defenses such as vitamins C and E and beta carotene; it is thus difficult for these cells to protect themselves from free radical attack.¹

An ideal therapy for incurable degenerative brain diseases would protect the brain from free radical damage, maintain

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or enhance key neurotransmitter action and help the brain to function optimally. Huperzine A, a natural product isolated from the club moss *Huperzia serrata*, fulfills these requirements. Huperzine A (HupA) has been shown to be a promising agent for a wide range of memory and brain disorders, including Alzheimer's disease. It works by the following mechanisms.

Acetylcholine is a neurotransmitter that is essential for normal learning and memory function *in vivo* (in living organisms). Neurotransmitters carry chemical messages from one nerve cell (the presynaptic nerve) to the next, causing the receiving neuron (the postsynaptic nerve) to "fire" its neurotransmitters (see figure on page 16). Because nerve cells are close together (separated by a space called a "synaptic cleft") the released neurotransmitter (in this case, acetylcholine) will activate the next cell in the sequence. It will bind to a "cholinergic" receptor (one that binds acetylcholine) on the postsynaptic nerve. In the human and animal brain the enzyme "acetylcholinesterase" (AChE), normally breaks down acetylcholine, keeping it from repeatedly firing the nerve and thus regulating the availability of this neurotransmitter in the brain. Acetylcholine function is deficient in patients with memory impairments or Alzheimer's dementia, probably due to selective degeneration of acetylcholine-producing neurons in the brain. It has been demonstrated in Alzheimer's disease patients that inhibitors of AChE (also called acetylcholinesterase inhibitors) reduce the breakdown of acetylcholine, and thus increase its availability. This can conceivably lead to an improvement in the patient's condition. HupA is a potent inhibitor of AChE, and thus helps to increase levels of acetylcholine in the brain.

Short- or long-term administration of Huperzine A has been demonstrated to induce a potent inhibitory effect of high affinity transport of choline in the brain.² Choline is the starting material for the synthesis of acetylcholine. Inhibiting choline's transport allows more to be available for synthesis of acetylcholine (see figure on page 16).

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An important property of HupA is that there is no significant development of tolerance to it; it can be used continuously.² Tolerance develops in response to many of the effects of physostigmine, and in the case of tacrine, a second dose results in a varied response to the AChE inhibitor.

Scientific research has demonstrated the diverse therapeutic potential of HupA: it is a potent, selective and reversible inhibitor of AChE, with a longer duration of action than other AChE inhibitors and minimal side effects. HupA has been identified to have profound beneficial effects in the following areas:

- Learning and memory retention
- Improve focus and concentration
- Treatment of cognitive and memory impairment
- Improved nerve transmission to muscles
- Powerful and reversible long-term inhibitor of AChE activity in the brain
- Dementia resulting from strokes and senile or presenile dementia
- Improved clinical picture for patients with myasthenia gravis
- Improved short-term and long-term memory in patients with cerebral arteriosclerosis (hardening of arteries in the brain)
- Alleviation of symptoms related to glaucoma
- Prevention of organophosphate pesticide toxicity
- Prevents nerve gas toxicity
- A novel psychotherapeutic agent for improving cognitive function in Alzheimer's patients
- A superior safety and efficacy profile compared to other cholinesterase inhibitors

HupA has neuroprotective ability also: it can stop damage to nerve cells and can block the effects of glutamate toxicity. Glutamate is an excitatory (stimulatory) neurotransmitter. During a stroke or other brain injury, excess glutamate is released in the brain, causing inappropriate release of enzymes inside cells that leads to cell death. This protective

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ability of HupA may be helpful in treating strokes, epilepsy and other neurological disorders.

WHAT IS HUPERZINE A?

Huperzine A (HupA) is found in an extract from the club moss *Huperzia serrata*, which grows at high elevations and in cold climates. It has been used for centuries in Chinese folk medicine,³ and is also known as *Qian Ceng Tu*. In China it was used to treat fever and inflammation, and for the past several years has been a prescription medication for treatment of dementia. Researchers claim that it helps alleviate memory problems in the elderly, as well as in those individuals with Alzheimer's disease (AD). HupA, a *Lycopodium* alkaloid, was first isolated from *Huperzia serrata* (Thumb) Trev. (also known as *Lycopodium serratum* Thumb) at the Zhejiang Academy of Medicine and Shanghai Institute of Materia Medica, Chinese Academy of Sciences, China.⁴ Dr. Alan Kozikowski, Professor of Chemistry in the Neurology Department in Georgetown University's Drug Discovery Program, in Washington, DC, has studied it extensively, and is the researcher who first synthesized HupA. As he reported in *JAMA (The Journal of the American Medical Association)*, HupA has been used to treat an estimated 100,000 people in China, suggesting that it is safe to use.⁵ The use of AChE inhibitors to alleviate symptoms of Alzheimer's disease has been the most promising approach thus far in dealing with this unresponsive condition. HupA, a natural, potent, and selective cholinesterase inhibitor has proven superior to other acetylcholinesterase inhibitors now recommended for the management of Alzheimer's disease; its neuroprotective action is an added benefit. Scientists have thus demonstrated that HupA holds tremendous promise for improving the quality of life for people with a wide range of memory impairments, including Alzheimer's dementia.

ALZHEIMER'S DISEASE DEFINED

Alzheimer's disease (pronounced Alz'-hi-merz) is a progressive, degenerative disease that attacks the brain and results

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in impaired memory, thinking and behavior. Damage is irreversible. The disease was first described by Dr. Alois Alzheimer in 1906. This mental decline is related to a loss of nerve cells and the links (synapses) between them. Alzheimer's disease (AD) is the most common form of dementia. It is not part of the normal aging process and always represents a disease process. AD is the fourth leading cause of death in adults after heart disease, cancer and stroke. Men and women are affected almost equally.

Researchers have developed a deeper understanding of the changes that occur in the brain (plaques and tangles) and behavioral changes that characterize the disease. Identified risk factors are age and family history. Most people diagnosed with AD are older than age 65; however, AD can occur in people in their forties and fifties. The course of the disease varies from person to person, as does the rate of decline. On average, AD lasts from four to eight years after diagnosis; however, it can continue for up to twenty years.

The first symptoms of AD include loss of recent memory, faulty judgement and personality changes. In the disease's early stages, people with AD may forget how to do simple things such as washing their hands. Often, they can no longer think clearly or remember the words for familiar objects or people's names.

The causes of AD are not known and are currently undergoing rigorous scientific investigation. Suspected causes include multiple mini strokes, defective genes or a genetic predisposition, abnormal protein build-up in the brain (amyloid—see below) and environmental toxins.

Amyloid is a protein-like substance deposited between cells and found in many organs of the body in a wide variety of diseases. In Alzheimer's disease, one of the main abnormalities seen in the brains of patients with the disease are plaques made of tangled nerve cells surrounding a central amyloid core. Amyloid consists of fibrils (string-like chains of protein) which represent 95 percent of the substance. The other 5 percent is glycoprotein (a sugar joined to protein).

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There are several types of amyloid. Beta-amyloid protein (A β) is the type comprising the core of the plaques found in the brains of people afflicted with AD. It is derived from a much larger protein called amyloid precursor protein (APP). Normally, APP is cleaved (broken), releasing a fragment called APPsec. This fragment does not form amyloid. During abnormal processing of APP, as is found in AD, the A β (also called amyloid beta-protein) fragment is cleaved from APP. This results in the accumulation of A β which then forms amyloid. In cases where AD runs in families, the genetic defect can be found in the processing of APP. The fibrillar amyloid beta-protein has been implicated in the development of Alzheimer's disease due to its neurotoxicity and its ability to activate an inflammatory response in the brain.

The importance of decreasing the brain's inflammatory response is demonstrated by the fact that people with rheumatoid arthritis seem to be protected from developing Alzheimer's disease.⁴⁷ The theory behind this finding is that patients with arthritis are usually taking large amounts of anti-inflammatory medication. Although aspirin and non-steroidal anti-inflammatory drugs are not the best way to control inflammation, the fact that the inflammation has been reduced does make a difference in the incidence of AD. Any substance that reduces inflammation, such as HupA, will decrease the level of oxidative stress in the brain tissue.

WHY HUPERZINE A?

It has been demonstrated repeatedly that memory loss or impairments and cognitive dysfunctions are accompanied by a dramatic reduction in acetylcholine synthesis and/or release in the nerve cells.⁴⁸ Interest in the functions of the central cholinergic system (the nerves in the brain that use acetylcholine as a neurotransmitter) has been stimulated by the finding that a decline in function in this area may underlie part of the cognitive deterioration seen in normal and pathologic (disease-related) aging. Postmortem (at autopsy) analysis of the brains of patients with senile dementia of

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the Alzheimer type has shown a decline in central (brain) cholinergic activity that correlates with their mental test scores. As in the case of dopamine replacement (a treatment approach used in Parkinson's disease) therapeutic trials of cholinomimetics (compounds similar to acetylcholine) and cholinesterase inhibitors have been used with the goal of enhancing cerebral cholinergic nerve transmission. The finding of a severely damaged and underactive cholinergic system in the brain of patients with memory impairments and Alzheimer's dementia has encouraged such research.

Acetylcholinesterase inhibitors have been reported to improve memory in a number of patients suffering from Alzheimer's dementia in its early stages. It has also been demonstrated that these inhibitors increase secretion of the beneficial amyloid precursor protein (APP) in the brains of rats. APP, when processed normally, has been demonstrated to enhance the working and reference memory function as well as increasing the synthesis and release of acetylcholine in the brain. The two types of memory are discussed on page TK, in the Animal Studies section. Thus, the long-term use of cholinesterase inhibitors such as HupA not only improves cholinergic function, but an increase in normal APP metabolism will further help patients suffering from memory impairments including Alzheimer's dementia.

The main reason for studying cholinesterase inhibitors such as HupA, therefore, is that these compounds will increase extracellular acetylcholine levels. Based on the available experimental and clinical information,¹⁴ an ideal cholinesterase inhibitor suitable for symptomatic treatment of memory and cognitive impairment including Alzheimer's dementia should satisfy the following requirements:

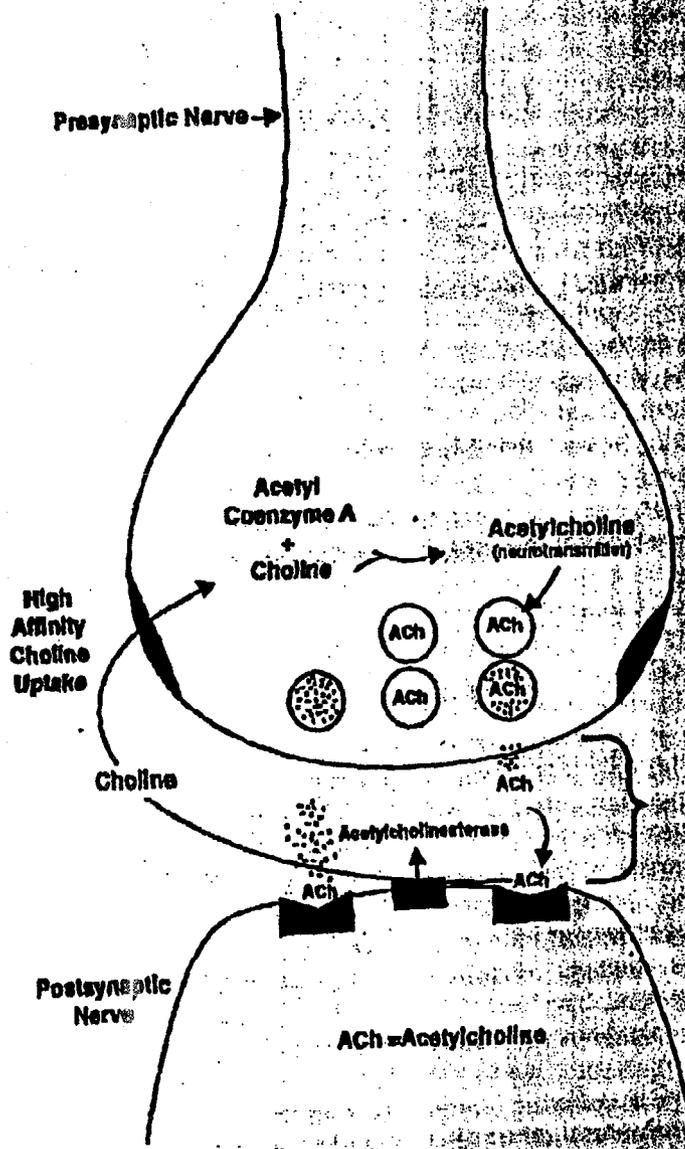
- a) Produce a long-term reversible acetylcholinesterase inhibition in the brain with increased synthesis or release of acetylcholine in the brain;
- b) Not inhibit acetylcholine synthesis or release in nerve endings; and
- c) Produce only mild side effects at therapeutic doses.

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Huperzine A fulfills all of these requirements. It is an excellent psychotherapeutic agent, a potent, selective and long-term reversible inhibitor of acetylcholinesterase^{10,12} and it induces the synthesis and release of acetylcholine. It also has a long resident time of interaction with acetylcholinesterase which may make it more effective. Its long half-life means that it can be given at lower dosage and fewer times per day than inhibitors with shorter half-lives. Half-lives are discussed further on page TK. With the compound physostigmine, AChE is strongly (80 percent) inhibited in the brain within a few minutes; but the effect lasts only 60 minutes.¹³ With HupA the inhibition is maintained for six hours after one dose. HupA also possesses unique blood-brain barrier penetration (it reaches its site of action) with minimal side effects at the therapeutic dose.

Other AChE inhibitors range from some of the most toxic agents to ever be synthesized (VX, Sarin, Soman) to therapeutic agents that are useful in the treatment of glaucoma (physostigmine) and myasthenia gravis (neostigmine). Only a few cholinomimetic compounds, physostigmine (also called eserine), tacrine and donepezil, have been evaluated extensively in dementia on a large scale, although there are more therapeutic candidates in various states of study. So far there have been problems with some of the compounds. The therapeutic effect of physostigmine, for example, is limited by its short duration of action, narrow therapeutic window and peripheral cholinergic effects. The term "narrow therapeutic window" means that the drug becomes toxic at doses above that needed to be effective. Drugs that have peripheral cholinergic effects will act on nerves outside the brain, causing side effects. For example, there may be fasciculations (involuntary contractions, or twitchings, of groups of muscle fibers in the muscles in the limbs). The most frequent and important side effect of tacrine is hepatotoxicity (liver toxicity), which limits its clinical value. Tacrine does not appear to alter the neurodegenerative disease; unlike HupA it is not neuroprotective. It also does not provide any benefit in non-Alzheimer's dementia.

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Huperzine A Figure

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Compared to physostigmine and tacrine, HupA is more potent at inhibiting acetylcholinesterase both *in vitro* (in the laboratory) and *in vivo*; it is about three times more potent than physostigmine.¹⁸ HupA also persists in the body for a longer period of time than physostigmine. At therapeutic doses, the side effects of HupA were minimal when compared with those caused by physostigmine and tacrine.

MECHANISM OF ACTION

Huperzine A works by a unique mechanism that has been scientifically discovered and reported in many research journals. It is a potent acetylcholinesterase inhibitor. Acetylcholine is the neurotransmitter in the brain that is responsible for carrying electrical impulses from one nerve to another (see appendix). It is made in the end section of nerve fibers and packaged into small vesicles where it is stored until released (see figure). Once acetylcholine has been secreted by the nerve ending, it persists for a few seconds. In a normal brain, the enzyme acetylcholinesterase serves a housekeeping function by breaking down the acetylcholine. It is split into an acetate molecule (from the "acetyl" part) and choline. The choline (a member of the B-vitamin family) is then transmitted back into the nerve ending to be used again to make acetylcholine. The brains of people with Alzheimer's demonstrate a deficiency of acetylcholine because of damage to the nerve cells that secrete it. Even with this deficiency, the acetylcholinesterase enzyme keeps working to get rid of whatever acetylcholine is released from the damaged nerve cell. This creates a deficiency. Huperzine A stops this enzyme from breaking down acetylcholine, thus preventing deficiency and improving mental function.

The finding of a severely damaged and underactive cholinergic system in the brains of patients with memory impairments and Alzheimer's dementia has led to clinical trials of new cholinomimetics including cholinesterase inhibitors. It has been demonstrated repeatedly that memory loss or impairments and cognitive dysfunctions are accompanied by dramatic reduction

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in acetylcholine synthesis or release in the nerve cells. Investigating acetylcholine release is one way of testing the function of cholinergic synapses (the transmission of acetylcholine across the gap to the next nerve cell). It can be done with different drugs or compounds in animal or human autopsy tissue slices⁴ using techniques that measure extracellular acetylcholine (amounts outside the cell)⁷—an important way to assess drug effects on the body. Acetylcholine release is governed by complex factors such as membrane integrity and cholinergic receptor biochemistry. Receptors, such as those for acetylcholine, will function to different degrees depending on factors such as heredity (i.e., fewer insulin receptors increase the probability of getting diabetes) and adverse conditions during early childhood when the brain is still developing (some types of schizophrenia).

All cells, including nerve cells, are surrounded by a cell membrane. This membrane allows materials such as nutrients, hormones and neurotransmitters into the cell and allows cell products or waste material to leave the cell. If the integrity of this membrane is not maintained, normal function will be impaired. Cell membranes can be degraded by free radicals, or can stiffen because of a deficiency in the essential fats. Cell membranes contain good fats such as omega-3 fatty acid and oleic acid; without them cell walls harden and transport in and out is decreased or stopped.¹³ Cells die as a result. Huperzine A protects cell membranes from the effects of free radicals, with the result that nerve cells do not die as quickly, or in as large numbers as with the absence of such protection.

THE CHEMISTRY OF HUPERZINE A

The chemical name for Huperzine A is (5R, 9R, 11E)-5-amino-11-ethylidene-5,6,9,10-tetrahydro-7-methyl-5,9-methano-18 / HUPERZINE A: BOOST YOUR BRAIN POWER

cyclooctenol[pyridin-2 (1H)-one. It is easy to understand why its name was shortened. The longer name describes the chemical structure of the molecule (see appendix). Although a lesson in chemistry is beyond the scope of this Guide, some of the facts about its structure are worth mentioning. For HupA to inhibit AChE, it must interact with it and prevent the enzyme from working. To accomplish this successfully, HupA contains various chemical groups and interactive abilities that give it a high affinity for AChE.¹⁴ Thus, the chemical structure of HupA allows it to maintain its unique ability to reversibly inhibit acetylcholinesterases.¹⁷

The chemical stability of Huperzine A is excellent. It is resistant to structural changes when placed in acidic or alkaline solutions.⁶ Furthermore, long-term incubation of HupA with acetylcholinesterases at 24°C (75°F) resulted in no detectable changes in the chemical structure of HupA.⁴ The stability in various solutions and the persistence of its effectiveness against AChE at different temperatures indicates that HupA will persist longer in the body and that tablets or capsules containing HupA will have a longer shelf life. Sophisticated laboratory techniques allow the determination of the exact quantity of HupA in an extract of *Huperzia serrata* (see appendix).

Regarding the structure and function of HupA, Joel Sussman, Ph.D., Professor of Structural Biology at the Weizmann Institute of Science in Rehovot, Israel, says that HupA can bind to AChE better than other AChE inhibitors.⁵ Dr. Sussman stated in an article published in the *Journal of the American Medical Association (JAMA)*:⁵ "It is as if this natural substance were ingeniously designed to fit into the exact spot in acetylcholinesterase where it will do most good."

The three-dimensional structure of the complex between HupA and AChE was worked out by a Weizmann Institute graduate student, Mia Raves, along with her professors at Weizmann and with Dr. Alan Kozikowski of Georgetown University (formerly at the Mayo Clinic, Jacksonville, FL).¹⁶

HupA has a longer half-life compared to the AChE inhibi-

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tors physostigmine, and tacrine (Cognex®). The half-life is the time during which half of the drug or compound has been metabolized (by breakdown and excretion from the body). The longer half-life means that the drug stays in the body longer and that less of it has to be given (and less often) than a similar compound with a shorter half-life. This characteristic is especially beneficial if the compound has few side effects, as is the case for HupA. The half-life for HupA is 288.5 minutes (4.8 hours), while for physostigmine it is twenty minutes.¹⁹ Tacrine has a half-life of 2.7 hours.

According to Dr. Kozikowski the longer half-life and the fact that the complex of HupA and AChE dissociates (breaks apart) slowly may indeed make it a more effective therapeutic agent. Also, the strong selectivity of HupA for AChE suggests that HupA will have fewer side effects than tacrine or donepezil. A compound that acts very specifically, as does HupA, will do only the job it is intended to do. Compounds that are not as specific will bind with other proteins in the cell (non-specifically) as well—interfering with various cellular reactions. This results in adverse side effects from the unwanted interaction, i.e., nausea, vomiting, salivation and sweating. HupA can produce some of these side effects when given in high doses, but at therapeutic doses it produces very few. Donepezil (Aricept® or E2020) with a 72-hour half-life may cause bradycardia (slowing of the heart beat), which could be a problem for patients with heart disease. Tacrine causes elevation in liver enzymes (an indication of liver damage) in about half of treated patients, and weekly blood monitoring is needed; donepezil does not appear to affect liver enzymes.

Synthesis of Huperzine A

Because Huperzine A is difficult to procure in large quantities from natural sources, attempts were made to synthesize HupA in the laboratory. A racemic mixture of HupA and a variety of its chemical structural analogs (molecules with similar structure and function) have been synthesized by Kozikowski and colleagues.^{19,20} A racemic mixture means that there

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are two forms of the molecule—one active and one not. When scientists try to copy natural molecules in the laboratory, the racemic mixture is easiest to synthesize. Separating the two molecules in the racemic mixture is difficult and expensive, so the inactive form is usually just left in as part of the finished product. Such a product will not be as active as the original substance because of the presence of the inactive molecule. In fact, the synthetic racemic mixture of HupA was found to be three times less potent than natural HupA.²¹ The Food and Drug Administration (FDA), however, required that the inactive form be removed from the product. Dr. Kozikowski accordingly synthesized an "optically pure" form of HupA—one that contains only the active molecule. This optically pure form can be found in tablet form.

Whenever a therapeutic compound is developed, researchers always try to improve it by adding different chemical groups at various places on the molecule. Sometimes this works and a more effective compound (analog) is produced. In the case of HupA, however, none of the analogs proved as potent as the original, natural parent compound.

Both natural and synthetic HupA were shown to be more potent than physostigmine (a drug used to enhance memory in patients with Alzheimer's disease) as inhibitors of acetylcholinesterase *in vitro*. Moreover, this inhibitory effect on acetylcholinesterase *in vivo* was of a longer duration (peak activity of 20 minutes for physostigmine versus 60 minutes for the Huperzine A variants). These results indicate a similar biological mechanism of action between the two, but that synthetic racemic HupA exhibits a weaker biological activity than the natural product.²²

PHARMACOKINETICS OF HUPERZINE A

The process by which a drug or other compound is absorbed, distributed, metabolized and eliminated by the body is called pharmacokinetics. Understanding the pharmacokinetics of a drug, nutritional supplement or food in the human body is very important to understanding the characteristics of that

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agent as it acts in a biological system. An ideal therapeutic agent will be absorbed rapidly, distributed widely, produce biological or therapeutic efficacy, and be eliminated at a moderate rate. It will also cause minimal or no toxicity in the body. The pharmacokinetics of HupA have been investigated in rodents and human volunteers. In these studies, HupA was absorbed rapidly, distributed widely in the body and eliminated at a moderate rate. In mice, 15 minutes after intravenous (IV) administration of HupA labeled radioactive for ease of detection, the HupA concentration was highest in the kidney and liver, moderate in the spleen, lung and heart, and lower in the brain. In pregnant mice, a small amount of detectable (radioactive) HupA was found in the fetus after administration. The majority (73 percent) of the radioactivity was excreted in the urine 24 hours after IV administration, while only 2.4 percent was recovered from feces.¹⁹ In a similar study, Tang and colleagues²³ have demonstrated that 60 minutes after IV injection, the drug is present in all brain regions but is particularly concentrated in certain areas such as frontoparietal and striatal regions of the cerebral cortex. It is these cortical areas that are low in acetylcholine in the brains of patients with Alzheimer's disease, so this specifically may indicate a therapeutic advantage of HupA use. It was also noted that lower doses of HupA were needed in rats to produce inhibition of AChE than with other compounds. If this proves true in humans, it may not be necessary to give high doses to produce a therapeutic effect. The result would be fewer side effects.

PRECLINICAL AND CLINICAL STUDIES WITH HUPERZINE A

Animal Studies

Extensive laboratory testing of HupA has been done. The Ames mutation test (done on bacteria) was conducted using increased dosages of HupA. When a compound is toxic it will cause abnormalities or mutations in bacteria which either affect the rate of growth of the bacteria or kill them. Such a substance is termed a "mutagen." HupA was compared with

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cyclophosphamide (a known mutagen). The test results indicated no noticeable differences between Huperzine A and a group of untreated bacteria that spontaneously mutate.

Teratological tests that determine the effect of a compound on offspring of animals given the substance were done in mice and rabbits. No external, internal organ or skeleton deformities were observed for any of the dosages.

The therapeutic index of HupA was also determined. This property is defined as the ratio of dose required to produce a toxic effect versus the dose needed to give the desired therapeutic response. The therapeutic index of a drug is also an approximate statement about its relative safety. The larger the ratio, the greater its relative safety.

Dr. Yan and colleagues²¹ determined the comparative therapeutic indices of five leading acetylcholinesterase inhibitors including Huperzine A, Huperzine B (also isolated from *Huperzia serrata*), neostigmine, physostigmine and galanthamine in mice and rats. HupA was compared for its biological efficacy and potency in various tests using isolated animal muscle tissues. The relative order of magnitude of the therapeutic indices (and safety order) of these different compounds was, in mice, Huperzine B (26.5) > Huperzine A (23.1) > neostigmine (8.6) > physostigmine (3.8), and in rats, the relative magnitudes were Huperzine B (294.8) > Huperzine A (72.9) > galanthamine (36.0) > neostigmine (34.0) > physostigmine (7.2). HupA was given in a lower dose than HupB. Based on these findings the researchers recommended that Huperzine A and Huperzine B should be of therapeutic value in the treatment of various peripheral or central nervous system diseases manifested by a cholinergic hypofunction (low acetylcholine levels). HupA would be the choice over HupB because less is used and side effects are thus fewer.

A similar toxicity comparison has also been reported by Tang²⁵ in mice using different cholinesterase inhibitors including Huperzine A, tacrine, physostigmine and galanthamine (table 1). In the table the therapeutic dose is compared with the LD₅₀. The LD₅₀ is the dose at which 50 percent of the experi-

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mental animals would die. The products that are the safest will have a therapeutic dose that is much lower than the LD₅₀.

Table 1. Biological Efficacy and Safety of Acetylcholinesterase Inhibitors in Mice

| Compounds | Therapeutic Dose (mg/kg, oral) | LD ₅₀ (mg/kg, oral) |
|---------------|--------------------------------|--------------------------------|
| * Huperzine A | 0.2 | 4.6 |
| Tacrine | 16.0 | 53.1 |
| Physostigmine | 0.3 | 1.96 |
| Galanthamine | 2.0 | 27.1 |

mg = milligrams

kg = kilograms of body weight

LD₅₀ = the dose at which 50 percent of recipients are dead

HupA is safe because it would take more than 20 times the therapeutic dose to reach the LD₅₀.²⁵ This is determined as follows.

$$\frac{LD_{50}}{\text{Therapeutic Dose}} = \frac{4.6}{0.2} = 23$$

Example: If the therapeutic dose was one milligram, it would take 23 milligrams to reach toxicity.

As an example, for tacrine, that number would be 3.3. In other words, taking just 3 milligrams instead of one milligram would result in the death of 50 percent of the test population.

Huperzine A failed to cause liver toxicity in dogs and rabbits or any other side effects such as nausea, vomiting, gastrointestinal upset, depression, etc., which are common following physostigmine treatment. These data demonstrate the relative safety of HupA as compared to other cholinesterase inhibitors. In most human clinical trials, HupA was used at low therapeutic doses and no adverse side effects have been reported.

The effect of Huperzine A was shown to be more potent in improving memory impairments than with the AChE inhibi-

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tors E2020 (aricept or donepezil) and tacrine.²⁶ The memory impairments induced by scopolamine, a drug that produces amnesia similar to that in Alzheimer's disease, were evaluated using a maze task. Both working and reference memory were affected by scopolamine. Working memory refers to tasks that were first learned. Reference memory is the ability to recall a task learned in the past. For example, if a rat was being tested for its ability to run through a maze and find the bait, there would be a deficit in working memory if the rat went back to the same maze corridor where the bait had just been found. If a rat ran first into an unbaited maze, this would be an error in reference memory. Re-entry into an unbaited maze would represent a deficit in both types of memory.

In this experiment, to compare the inhibition of AChE by HupA with the effects of E2020 and tacrine, the scopolamine dose (0.2 mg/kg) significantly impaired spatial memory in rats. Huperzine A (0.1-0.4 mg/kg, orally), E2020 (0.5-1.0 mg/kg, orally) and tacrine (1.0-2.0 mg/kg, orally) reversed these scopolamine-induced memory deficits. Huperzine A was found to be the most selective acetylcholinesterase inhibitor. It improved the working memory deficit and amnesia induced by scopolamine significantly better than E2020 or tacrine. The action of the three AChE inhibitors was also tested in isolated mouse muscle.²⁷ It is easier to measure the effects of these inhibitors at the neuromuscular junction (the place where nerve and muscle meet) than in the synapses between nerve cells in the brain. It was found that HupA was more effective in inhibiting AChE than tacrine as shown by a decrease in muscle movement. Although E2020 (aricept, donepezil) had a stronger effect on the muscle than HupA, the E2020 was less selective. Such a decrease in specific inhibition of AChE may result in increased side effects. The researchers in both studies stated that the results confirm that HupA is a promising agent to evaluate for clinical therapy of cognitive impairment in patients with Alzheimer's dementia.

The effect of Huperzine A on maze performance in rats was assessed by Drs. Xiong and Tang²⁸ in a comparison with phy-

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physostigmine. The maze is a valuable apparatus in the study of spatial memory. It offers an alternative that closely resembles the natural food-seeking behavior of species such as rats. The rats were trained to run in a spatial, radial arm maze using a procedure to determine the two memory functions (working and reference memory). The rats were trained so that baseline error rates were low (they practiced beforehand), which permitted the deficit in reference memory to be observed. Both scopolamine and mecamylamine have been reported to disrupt both the working and reference memory of rats.²⁰ Low doses of scopolamine (0.125, 0.15 and 0.2 mg/kg, intraperitoneally or into the body cavity, 30 minutes before a session) impaired both working and reference memory in the radial maze. In contrast to scopolamine, mecamylamine (5, 10 and 15 mg/kg) did not cause any deficit in working and reference memory. Huperzine A (0.1, 0.2 and 0.3 mg/kg, intraperitoneally, 20 minutes before testing) and physostigmine (0.3 mg/kg, intraperitoneally, 20 minutes before testing) could reverse scopolamine-induced deficits in the task. Huperzine A completely reversed the scopolamine-induced deficit of maze performance. The memory improvement achieved with Huperzine A was comparable to that produced by physostigmine. Huperzine A exhibited a wide dose range (it worked at many different doses) for decreasing the scopolamine-induced memory impairment. Long-term treatment with Huperzine A (0.25 mg/kg, orally, once a day) for eight consecutive days was as potent as immediate high dose treatment in reducing scopolamine-induced amnesia.

The study further indicated that there was no significant tolerance to the memory-improving effect of Huperzine A. This is important because tolerance develops when physostigmine is used. This finding was consistent with the study conducted by Laganier and colleagues, who reported that Huperzine A-induced inhibition of acetylcholinesterase activity was as potent long-term as it was after immediate treatment.² These researchers have further demonstrated that following *in vivo* treatment with Huperzine A there was no decrease in turnover

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of acetylcholine in the brain (it remained available for use). HupA also maintained long-lasting levels of acetylcholine for neurotransmission in the brain. Laganier and colleagues concluded that HupA may be more effective and less toxic than physostigmine. This would be especially true in diseases where long-term inhibition of AChE is required. This is the case with Alzheimer's disease.

The effects of Huperzine A were assessed on the performance of rats treated with a specific cholinergic neurotoxin, AF64A.³¹ This neurotoxin was used to treat rats before the animals entered the radial maze. AF64A is known to disrupt the working memory processes by altering cholinergic brain function. AF64A caused significant impairment in a rat's ability to perform the necessary spatially-oriented tasks needed to succeed in the maze. The behavioral impairment was associated with a significant decrease in the activity of mechanisms requiring acetylcholine in the hippocampus, which is a portion of the brain highly involved in neurotransmission animals and humans. Huperzine A significantly decreased the AF64A-induced memory deficit and improved cholinergic function. There were no side effects.

These animal experiments demonstrate that Huperzine A is a highly promising therapeutic agent in alleviating neurological disorders including short- and long-term memory dysfunctions and Alzheimer's dementia.

Human Studies

Administration of acetylcholinesterase inhibitors is a major pharmacological approach currently employed in the treatment of Alzheimer's disease. This strategy is used with the purpose of inhibiting acetylcholine degradation *in vivo*, and hence alleviating the cholinergic deficiency, which has been shown to occur in patients with Alzheimer's disease. Human studies (clinical) have been conducted to examine the effects of HupA.

The pharmacokinetics of Huperzine A tablets were monitored in both human male and female subjects aged 27 ± 6 years and weighing 58 ± 7 kg.¹⁵ All volunteers were healthy.

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and not pregnant or menstruating. The volunteers were given a tablet containing 0.09 milligrams of HupA two hours before breakfast. Huperzine A levels in the blood plasma of these volunteers were then determined by sophisticated laboratory techniques. It was found that AChE was inhibited for 288 minutes with HupA, while inhibition lasted 20 minutes for the AChE inhibitor, physostigmine. These data demonstrate that Huperzine A was released and rapidly absorbed *in vivo*, widely distributed in the body and eliminated at a moderate rate. Based on these findings the researchers indicated that it would be best to take HupA in tablet form two to three times a day.

A recent clinical study was conducted by Dr. Xu and colleagues²¹ at Zhejiang Medical University, Shanghai, China, in patients with Alzheimer's disease. Using a multicenter, prospective, double-blind, parallel, placebo-controlled and randomized method, 50 patients were administered Huperzine A (200 micrograms in 4 tablets, orally) twice a day, and 53 patients were given a placebo (a "sugar pill") for eight weeks. All subjects were evaluated using the Wechsler memory scale, the Hasegawa dementia scale, the mini-mental state examination scale, the activity of daily living scale and the treatment emergency symptom scale. These are the "gold standard" measurement tests for cognitive function. About 58 percent of patients treated with Huperzine A evidenced improvements in their memory, cognitive and behavioral functions. The efficacy of HupA outperformed the placebo by 36 percent. Improvements noted by the patient's family members are shown in the following table. A significant difference was found between the two groups:

Table. Number of reports of Patient's Behavior from Family Members.

| Observation | Placebo (53 patients) | HupA (50 patients) |
|--------------------|--------------------------|-----------------------|
| Clear headed | 13 | 26 |
| Memory improving | 8 | 16 |
| Language improving | 2 | 6 |
| Unchanged | 34 | 21 |
| Total changes | 57 | 71 |

No adverse side effects were observed and the overall efficacy of HupA was higher than the placebo.

These studies are very significant because they demonstrate a dramatic improvement in memory following administration of Huperzine A to patients with age-associated memory impairments and disorders. Compared to other cholinesterase inhibitors, Huperzine A possesses a clearly superior safety and efficacy profile. Furthermore, the comparatively long duration of action of Huperzine A and minimal side effects make it a potentially useful therapeutic agent.

HUPERZINE A AND HEALTH BENEFITS

Neuropsychiatric Illness and Alzheimer's Dementia

The recognition of Alzheimer's disease as the fourth leading cause of death in the United States in adults (after heart disease, cancer and stroke), combined with its profound morbidity (diseased state), has led to the development of therapeutic strategies aimed at reducing the effects of, if not preventing, this disorder. The cost of caring for Alzheimer's patients—including the cost of diagnosis, treatment, nursing home care and formal or paid care—is estimated to be more than \$100 billion per year. The federal government covers \$4.4 billion and the states \$4.1 billion. Much of the remaining costs are borne by patients and their families. A therapy that could prevent, or even slow the progression of this disease, would surely be welcomed by health professionals and families.

Cholinergic mechanisms play a major role in controlling cerebral blood circulation and cerebral blood flow. These

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Alzheimer's disease (AD) is a slowly worsening brain disease. AD is marked by changes in behavior and personality and by a decline in thinking abilities that cannot be reversed. This mental decline is related to a loss of nerve cells and the links between them. The course of the disease varies from person to person, as does the rate of decline. On average, AD lasts from 4 to 8 years after diagnosis, however, it can continue for up to 20 years. AD advances in stages, from mild forgetfulness to severe dementia, or mental decline. Signs (or symptoms) of AD appear most often between the ages of 60 and 70. The first symptoms include loss of recent memory, faulty judgment and personality changes. Early in the disease, people with AD may forget how to do simple things like washing their hands. Often, they can no longer think clearly or remember the words for familiar objects or people's names. Later on, people with AD lose all reasoning abilities and become completely dependent on other people for their care. Patients often live for years. The disease eventually becomes so debilitating, however, that patients are likely to develop other diseases. Most commonly, AD patients die from pneumonia.

mechanisms depend on adequate amount of acetylcholine. Acetylcholine can act to dilate or constrict blood vessels, depending upon where in the brain it is released. A deficiency in acetylcholine will prevent proper regulation of blood vessel diameter and result in impairment of brain function. The destructive process occurring in the brain of an Alzheimer's patient will worsen when an adequate blood supply to the area is not maintained. As the loss of cholinergic function progresses, memory becomes increasingly impaired. This impairment results in major neuropsychiatric dysfunction in the elderly.

The degree and extent of cholinergic deficit in this type of senile dementia correlates with the severity of cognitive impairment: the more pronounced the acetylcholine deficiency, the worse the cognitive function. Since a deficiency in the cholinergic system is believed to constitute one of the hallmarks of Alzheimer's dementia, reversible inhibitors of acetylcholinesterase (the enzyme that breaks down acetylcholine) that make their way into the central nervous system

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may serve as palliative agents in the treatment of the disease.

Studies using cholinesterase inhibitors with the goal of alleviating cholinergic deficiency have shown the most encouraging results in Alzheimer's dementia to date. Huperzine A gained considerable interest because of its unique anti-acetylcholinesterase activity and biochemical properties. As discussed earlier, its long half-life, unique blood-brain barrier penetration, minimal side effects, and high therapeutic indices suggested that Huperzine A may work better than existing drugs, including physostigmine and tacrine, for the treatment of Alzheimer's disease.

There is a considerable pharmacological evidence that the central (brain) cholinergic function plays an important role in learning as well as for the working and reference memory functions. Postmortem (autopsy) analysis of the brains of patients with senile dementia of the Alzheimer type has identified a decline in central cholinergic activity that correlates with their previous mental test scores. Interest in the functions of the central cholinergic system has been stimulated by the hypothesis that decline in this system may underlie the part of the cognitive deterioration seen in the normal as well as pathologic (disease-induced) aging brain. Huperzine A, considered one of the new generation of acetylcholinesterase inhibitors, has gained considerable notice because of its unique anti-acetylcholinesterase activity as well as memory-enhancing effects in a broad range of behavioral models. Huperzine A exhibits significant inhibition of acetylcholinesterase activity in all brain regions tested including hippocampus, striatum, hypothalamus and frontal cortex. Structures in the hippocampus regulate behaviors such as rage, passivity, learning and motivation. The striatum is closely connected to the amygdala, a part of the brain associated with fear, confusional states, disturbances of awareness and amnesia. The involvement of the amygdala in emotional states has important bearing on several forms of dementia. In Alzheimer's, the amygdala suffers from se-

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vere neuronal loss; this part of the brain shrinks. This is accompanied by marked memory impairment and changes in emotional behaviors, including a loss of spontaneity. The hypothalamus regulates sleep, food and drink intake, and endocrine (hormonal) functions throughout the body. The frontal cortex is the area of higher cognitive functions: learning and memory.

Learning Ability, Memory Enhancement and Cognitive Performance

In a series of behavioral studies, Huperzine A was found to improve cognitive performance in a broad range of animal models involving mice, rats and monkeys with induced amnesia.³³⁻³⁶ HupA also markedly improved the retention of a learned task when tested 24 hours later in aged mice. Enhancement of learning and memory performance, increased retention and faster retrieval processes were observed. The loss of cholinergic neurons in the brain has been demonstrated to be part of the aging process itself. This loss is considered an important element in the process of memory loss or memory impairment including dementia. Huperzine A improved cholinergic function by inhibiting acetylcholine degradation in the brain.

In other behavioral experiments, Huperzine A has been shown to improve mice and rats' performance in running through mazes,³⁷⁻³⁹ and to protect young and aged animals against sodium nitrite, scopolamine, cycloheximide, carbon dioxide-treated and electroconvulsive shock-induced disruption of a passive avoidance response.^{40,41} It also improved accuracy of memory in squirrel monkeys.⁴² These studies imply that Huperzine A is effective in a variety of classical behavioral tests designed to test an animal's learning ability and memory function. The duration of improved effects on learning and memory retention processes with oral Huperzine A were longer than that obtained with physostigmine, tacrine and galanthamine, the existing acetylcholinesterase inhibitors.

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Huperzine A and Muscle Contraction

Huperzine A, as discussed earlier, potentiates the skeletal muscle contraction and increased muscle tone in rats.⁴⁴ Clinically, in another study, Huperzine A was shown to improve muscle weakness significantly as well as memory in patients with impaired memory.⁴⁵ Because Huperzine A prevents the selective degeneration of acetylcholine-producing neurons in the brain and enhances the availability of acetylcholine in the brain of patients suffering from neurological disorders including Alzheimer's dementia, the connections between nerves and muscles function better. The improvement in neuromuscular cholinergic transmission leads to an improvement of the patient's condition.

More Memory Improvement

The study conducted in human subjects to assess improvement in muscle function using Huperzine A, also showed favorable effects in the treatment of age-related memory impairment.⁴⁶ In another comparative study with Hydergine[®] (a vasodilator, 600 micrograms), 30 micrograms of Huperzine A (given intramuscularly) appeared to improve memory for 1-4 hours after it was given to 100 aged individuals (ranging in age from 46-82; sex: 54 males, 46 females) suffering from memory impairment.⁴⁶ In this population of 100 subjects, 83 had no demonstrable brain disease but were suffering from age-related amnesia or memory dysfunction, and only 17 had probable Alzheimer's disease. Note that Alzheimer's disease cannot be definitely diagnosed before death. It is only at autopsy that the determination can be made with precision. The results of this experiment were very encouraging. Minimal or no side effects were observed.

A more comprehensive study was later conducted by Zhang and colleagues.⁴¹ The therapeutic effects of Huperzine A were studied by the random, matched and double-blind method (a scientifically valid and accepted method) on 56 patients with multi-infarct dementia (resulting from repeated small strokes). The patients were age 64 ± 7 years;

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there were 52 males, 4 females. Also studied were patients with senile dementia and 104 patients of senile and presenile simple memory disorders (age: 63 ± 7 ; sex: 58 males, 46 females). Each group was divided into two smaller groups—one given HupA with the other serving as a control group. The control groups were treated intramuscularly with only saline (salt) solution. The intramuscular dose of Huperzine A for multi-infarct dementia was 50 micrograms twice a day for four weeks, whereas that for senile and presenile simple memory disorders was 30 micrograms, twice a day for two weeks. HupA is also available and effective in oral (tablet) form. The Weschler memory scale was used to determine whether there was improvement of memory function in these patients. Huperzine A treatment significantly improved the memory of patients in both treatment groups (those that received the HupA) and did so significantly and with minimal observed side effects. Only a few patients felt slight dizziness and this did not affect the therapeutic effects.

Alleviation of Symptoms Related to Glaucoma

Glaucoma is a disease in which there is an increase in pressure within the eye. If the increase is high enough and persistent enough, there will be damage to the optic nerve and irreversible blindness can result. AChE inhibitors are used to alleviate this condition. Since Huperzine A can inhibit the transport of choline in the brain, this allows more to be available for synthesis of acetylcholine, the neurotransmitter important in maintaining normal eye function. It has been suggested that HupA may be a better therapeutic drug than other AChE inhibitors such as physostigmine, neostigmine or tacrine for the treatment of glaucoma.¹¹

Improvement of Symptoms in Myasthenia Gravis Patients

In a clinical trial, Huperzine A has been demonstrated to significantly improve muscle weakness associated with myasthenia gravis.¹² This is a neuromuscular disease character-

ized by weakness and marked fatigability of skeletal muscle. The defect caused by the disease involves nerve transmission at the neuromuscular junction, the area where nerve meets muscle. When a nerve "fires" and activates a muscle at the neuromuscular junction, the muscle should contract or move. Although patients with myasthenia gravis can initially move the muscle, repeated activation causes a diminished response and muscle movement cannot be maintained. AChE inhibitors, by keeping acetylcholine levels high, can increase muscle response. The study, conducted by Cheng and colleagues was done in 1986. The clinical effects of Huperzine A were compared with prostigmine, another AChE inhibitor. In 128 patients with myasthenia gravis, 99 percent demonstrated controlled or improved clinical symptoms of the disease. The duration of action of Huperzine A was 7 ± 6 hours. Side effects, except nausea, were minimal when compared with those induced by prostigmine.

Protection Against Nerve Agents and Pesticide Toxicity

Organophosphates, chemicals used as pesticides and nerve gases during wartime, are well known to enter the nervous system, react with cholinesterase, irreversibly inhibit it, and induce potential brain injury leading to coma and death. When AChE is irreversibly inhibited, there is no control over levels of acetylcholine. All the effects of acetylcholine will become exaggerated and continuous. In the eyes, the pupil will markedly constrict with pain and spasm of muscles; effects on the lungs include "tightness" in the chest and wheezing due to constriction of bronchial tubes, as well as increased mucus. In the gastrointestinal tract, nausea, vomiting, cramps, diarrhea and extreme salivation are produced. Death usually occurs by respiratory (lung) failure, often accompanied by a heart attack.

The remarkable selectivity for acetylcholinesterase and superior blood-brain barrier penetration ability of Huperzine A, along with its chemical stability and reversibility, suggests that it should provide a safe and long-lasting prophyl-

lactic treatment against nerve agent toxicity in humans. A comparative study against organophosphate toxicity using the nerve agent soman, was conducted in animals who were pretreated with Huperzine A or physostigmine (which is currently being used to prevent nerve agent toxicity). The study was designed to determine prophylactic (preventative) efficacy of Huperzine A in mice. Huperzine A dramatically prevented irreversible phosphorylation of the enzyme (AChE) by soman. As mentioned earlier, continuous activity of the enzyme results in dangerous and eventually fatal consequences. The effect of HupA against soman toxicity lasted a long time; this was consistent with the finding that HupA levels persist in the brain for a longer period than physostigmine. According to the researchers, the data indicated that HupA might provide significantly longer therapeutic activity than other AChE inhibitors used to manage diseases where there is a deficiency in cholinergic neurons. In this *in vivo* model Huperzine A demonstrated significant antidotal efficacy and much better protection compared to physostigmine.⁴⁴

CONCLUSION

The preceding sections describe how Huperzine A may serve as a highly promising therapeutic agent. Huperzine A has attracted considerable notice because of its unique, reversible anti-acetylcholinesterase potency and pharmacokinetic properties. The preliminary evidence indicates its potential clinical value in the treatment of dementia, myasthenia gravis and glaucoma. The potentially superior inhibition characteristics of Huperzine A, as compared to other cholinesterase inhibitors, have been attributed to the very slow rate of dissociation of the acetylcholinesterase-Huperzine A complex in solution.⁴⁵ Also, the interaction of Huperzine A with acetylcholinesterase appears reversible and does not result in any detectable chemical modification of the inhibitor. Reversibility is important because the enzyme (AChE) will not be turned on permanently (as is the case with nerve gases). Because HupA (the inhibitor) is not altered when it inactivates the enzyme, it can be released and act again. Huperzine A produces a long-term inhibition of acetylcholinesterase activity in the brain (up to 360 minutes) and increases acetylcholine levels up to 40 percent at 60 minutes.¹³

Physostigmine, another AChE inhibitor, produces a rapid effect (15 minute peak of 55 percent inhibition), but this effect is over within 120 minutes. After attaining peak plasma (blood) concentration in humans at approximately 30-60 minutes, physostigmine is cleared from plasma with a half-life of about thirty minutes.⁴⁷ The anticholinesterase action of Huperzine A in cholinergic synapses is stronger than that of tacrine (also known as tetrahydroaminoacridine).²⁷ The terminal half-life of Huperzine A is 4.8 hours. While the potency of Huperzine A is comparable to that of physostigmine and tacrine (a classical acetylcholinesterase inhibitor), its duration of action is 10- to 12-fold longer *in vivo* (up to 8 hours), and the incidence of side effects induced by Huperzine A is considerably less than that of physostigmine or tacrine. Tacrine has been approved by the FDA for the clinical treatment of patients with Alzheimer's dementia,²⁷ but the therapeutic usefulness of tacrine is limited by its liver toxicity. Huperzine A more significantly improved learning and memory in mice with higher efficacy than tacrine. In Phase II clinical trials, Huperzine A improved memory in Alzheimer's disease patients with minimal side effects.³² The Phase II clinical study with Huperzine A also showed that 99 percent of patients with myasthenia gravis were controlled and/or improved following administration of Huperzine A.²⁵ Furthermore, its long half-life, high oral bioavailability, unique blood-brain barrier penetration ability and safe therapeutic indices support the fact that Huperzine A is a potential candidate for treatment of memory deficits in patients with Alzheimer's disease. Huperzine A also has potential for treating other nervous system-related dementias as well

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as usefulness in protection against organophosphate pesticide or nerve agent toxicity.

ACKNOWLEDGMENT

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Qian

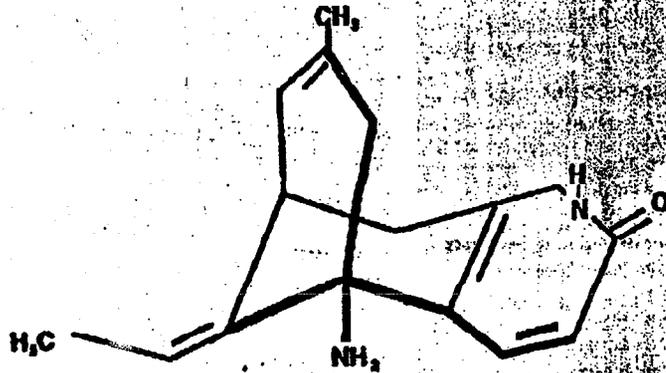
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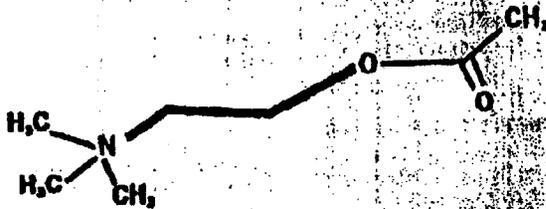
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Appendix



Huperzine-A



Acetylcholine

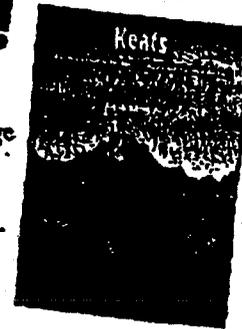
Chemical Structures of Huperzine A and Acetylcholine
 C=carbon
 H=hydrogen
 N=nitrogen

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HPLC Techniques for Quantitation of Huperzine A

Qian *et al.*¹⁶ has demonstrated a high performance liquid chromatography technique (HPLC) for the quantitation of Huperzine A. HPLC is a technique that uses a tube-like column filled with very small bead particles to separate the components of a liquid mixture of a substance, in this case the HupA extract. Depending on the molecular size of the component, it will either pass through the column or be trapped on the beads. By varying the size of the beads and the type of liquid flowing through the column, individual components in complex mixtures can be identified. The HPLC was equipped with an UV absorbance detector and a Spherisorb C₁₈ (150 mm x 5 mm I.D.; 5 μm particle size). The mobile phase was methanol: water (45:55, vol/vol) at a flow rate of 1 ml/min at 30°C column oven. The column effluent was monitored at 313 nm.

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REVIEW ARTICLES

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Edited by Denham Harman, Robin Holliday, and Mohsen Meydani

New York Academy of Sciences
New York, New York
1998

Double-blind Trial of Huperzine-A (HUP) on Cognitive Deterioration in 314 Cases of Benign Senescent Forgetfulness, Vascular Dementia, and Alzheimer's Disease

MA YONG-XING, ZHU YUE, GU YUE-DI, YU ZHEN-YAN, YU SAI-MEI,
AND YE YONG-ZHEN

Research Division of Aging and Antiaging, Shanghai Geriatric Institute,
Huadong Hospital, 221 West Yan An Road, Shanghai 200040, China

HUP, a new alkaloid extracted from *Huperzia serrata* (Thumb) Trev, by Liu, is a potent anticholinesterase with minimal toxicity. Tong has found that HUP may improve the learning and retrieval function of rats, and its facilitation actions were due to an effect on the central cholinergic system.

BENIGN SENESCENT FORGETFULNESS (BSF) TREATED WITH 0.03-0.05 mg HUP im b.i.d.

The first clinical trials used the double blind method on 120 patients with cognitive deterioration, with memory quotient (MQ) (WMS) < 100. The mean values of MQ of 60 treated and 60 controls were 76.27 ± 14.08 and 77.97 ± 12.55 ($p > 0.05$), respectively. The dosage was 0.03 mg im b.i.d. for 14-15 days. The mean values of MQ after the treatment (the interval between pre- and posttests of WMS with A and B form, respectively, is 1-2 months) of treated and controls were 91.85 ± 13.73 ($p < 0.01$) and 82.24 ± 15.10 ($p > 0.05$), with the MQ increase of 15.82 ± 10.02 and 4.40 ± 8.72 , respectively, ($p < 0.01$). The effective rates were 68.33 and 26.37% in the two groups. No significant side effects were observed.

The second trial included 16 patients of the HUP treatment group (0.03-0.05 mg im b.i.d. for 4 wks) with IQ (WAIS) < 105 (95.0 ± 7.6). The IQ increased to 100.7 ± 12 ($p < 0.01$) after the treatment. The value of IQ increase is 5.7 ± 0.68 as compared with 3.0 ± 0.36 in the hyperbaric oxygenation treatment group (2.5 ATA, 80', 4wks in 10 patients ($p < 0.01$)).

*BSF TREATED BY 0.1 MG HUP po q.i.d.

The clinical trials used the double blind method on 88 patients with cognitive deterioration, with MQ (WMS) < 100. The mean values of MQ of 44 treated and 44 controls were 82.8 ± 14.3 and 81.5 ± 14.4 ($p > 0.05$), respectively. The dosage was 0.1 mg po q.i.d. The mean values of MQ after the treatment (the interval between pre- and posttests of WMS with A and B form, respectively, is 2 months) of treated and controls were 93.5 ± 14.5 ($p < 0.01$) and 85.5 ± 16.5 ($p < 0.01$). The effective rates were 68.18 and 34.09% in the two

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groups. No significant side effects were observed except gastric discomfort (2), dizziness (1), insomnia (1), and mild excitement (1) in the treated group.

VASCULAR DEMENTIA AND ALZHEIMER'S DISEASE

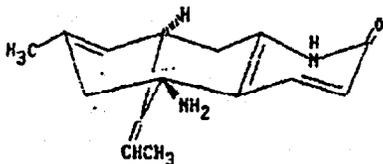
A clinical trial of vascular dementia (25) and Alzheimer's disease (55) was conducted on 40 treated and 40 control patients with the same dosage as for BSF. The MQ of the treated group increased from 50.40 ± 18.49 to 59.74 ± 18.73 ($p < 0.05$); that of the control group increased from 53.95 ± 14.74 to 55.85 ± 16.28 ($p > 0.05$). The MQ increase of 9.37 ± 10.38 is significantly higher than that of 1.90 ± 10.36 ($p < 0.01$). The effective rate of the treated group was 60%, significantly higher than that of 35% in the control group ($p < 0.05$). No significant side effects were observed except gastric discomfort or nausea (3) and dizziness (3) in the treated group.

It is concluded that huperzine is an effective and safe drug to improve cognitive and memory function in the aged and preaged.

HUPERZINE A

9*R*, 11*E*-5-Amino-11-ethylidene-5,6,9,10-tetrahydro-7-methyl-5,9-methanocyclooctenobipyridin-2(1*H*)-one
= 122-853

Cholinesterase Inhibitor
of Myasthenia Gravis
Antagonist of Acetylcholine



$C_{11}H_{16}N_2O$; Mol wt: 242.33
C 74.35%; H 7.49%; N 11.56%; O 6.60%

Isolation

Huperzine A is a new lycopodium alkaloid isolated from the Chinese folk medicine *Huperzia serrata* (L.) Trev. The powdered herb is extracted with 70% ethanol. The phenolic alkaloids are separated by treatment with dilute sodium hydroxide. The mixture is taken up on a basic silica gel column, and eluted with chloroform-methanol. Huperzine A is crystallized from acetone after evaporation of the solvent (2).

Description

Slightly yellow crystals, m.p. 229-30°; $n_D^{20} = 1.504$ ($d_4^{20} = 0.498$ MeOH). UV absorption: $\lambda_{max} = 231$ (log ϵ 4.01), 313 (log ϵ 3.893). IR ν_{max}^{KBr} : 3180, 1650, 1615, 1550 (1).

Pharmacological Actions

The anticholinesterase activity of huperzine A was studied *in vitro* (3). Rat erythrocyte membrane and kidney nuclei of pigs were used as sources of acetylcholinesterase, and rat serum was chosen as source of butyrylcholinesterase. The pI_{50} of huperzine A towards erythrocyte membrane and kidney nuclei was 7.2 and 7.9, respectively. The inhibiting effect was about 3 times as potent as that of physostigmine. Huperzine A was less potent than physostigmine towards rat serum. The alkaloid thus belongs to the class of mixed and reversible

cholinesterase inhibitors.

Rats were placed on an electrified grid in a Y-maze and learned to run into the light arm (safe area). The criterion of learning or retrieval was met after they had chosen the light arm 10 trials in succession. Huperzine A 100 and 167 μ g/kg i.p. administered 20 min before training caused a significant decrease in the number of trials to criterion. Facilitation of retrieval was also dose-dependent at doses of 36-167 μ g/kg i.p. Scopolamine 0.2 mg/kg s.c., atropine 5 mg/kg s.c. or hemicholinium 20 μ g/10 μ l i.c.v. antagonized the positive effects of huperzine A 0.1 mg/kg on retrieval process, but methylatropine 2 mg/kg s.c. did not do so. Under the same conditions, physostigmine 80-180 μ g/kg i.p. improved the learning and retrieval process, but neostigmine 30 μ g/kg i.p. did not. The facilitating actions of huperzine A were due to an effect on the central cholinergic system, especially on the muscarinic system (4).

Clinical Studies

One hundred twenty eight cases of myasthenia gravis were treated with huperzine A 0.4 mg i.m. once daily. Clinical manifestations were controlled or improved. Huperzine A showed a duration of action of 7 ± 6 h, whilst that of prostigmine was 4 ± 5 h (5).

The therapeutic effects of huperzine A and hyderygine were studied by a double-blind method in 100 aged patients with memory impairment. Memory was improved 1-4 h after injection of huperzine A, and the effect was sustained for about 8 h. The therapeutic value of huperzine A 30 μ g was superior to that of hyderygine 600 μ g (6).

Except nausea, the side-effects of huperzine A, such as fasciculation, dizziness, sweating, blurred vision, etc., were less and milder than those of prostigmine (5).

Manufacturer

Shanghai Inst. Materia Medica (China).

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4. Tang, X., Han, Y., Chen, X. and Zhu, X. Effects of huperzine A on learning and retrieval process of discrimination per

LORATADINE

Prop INN: USAN

Loratadine (Sp)

11-[N-(Ethoxycarbonyl)-4-piperidylidene]-8-chloro-6,11-dihydro-5H-benzo[5,6]cyclohepta[1,2-b]pyridine

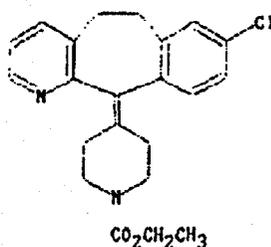
4-(8-Chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-1-piperidinecarboxylic acid ethyl ester

Sch-2985 1

[CAS-79794-75-5]

EN = 90-791

Antihistamine



C₂₂H₂₃ClN₂O₂, Mol wt: 382.89
 C 69.01%; H 6.05%; Cl 9.26%; N 7.32%;
 O 8.35%

Synthesis

This compound can be obtained by two different ways (Scheme 1):

1) By carboxylation of 8-chloro-6,11-dihydro-11-(4-piperidylidene)-5H-benzo[5,6]cyclohepta[1,2-b]pyridine (I) with ethyl chloroformate (II) in refluxing benzene (1, 2).

2) By reaction of 8-chloro-6,11-dihydro-5H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-one (III) with the Grignard reagent (IV) to give the tertiary carbinol (V), which is dehydrated with 85% H₂SO₄ affording 8-chloroazatadine (VI) (3). Finally compound (VI) is treated with ethyl chloroformate (II) in toluene (4).

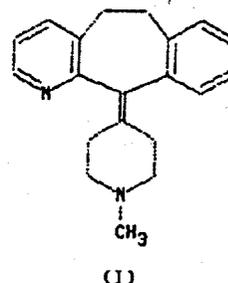
Description

Crystals, m.p. 128-30°.

(CES)

Introduction

Antihistamines became widely used in the mid to late 1940's. They quickly became established in the treatment of various allergic disorders, particularly rhinitis, conjunctivitis and urticaria. The problem of sedation limited the use of classical antihistamines and the search for H₁-antagonists without sedative potential has been a goal within the pharmaceutical industry. Several non-sedating H₁-antihistamines have been recently introduced in the clinic, others are under investigation and others are continuously emerging from patent literature (Table I). Recently, conversion of the basic tertiary amino function of the potent antihistamine azatadine (*Optimine*[®]) (1) to a neutral carbamate function led to the preparation of compounds which retained significant antihistamine activity, with little or no CNS effects. The most potent antihistamine, Sch-29851 (loratadine), in this series was selected for further evaluation in view of its lack of CNS side-effects (4).

**Pharmacological Actions**

Loratadine has been shown to be a potent antihistamine in laboratory animals and to have no CNS effects in a variety of animal species and test paradigms (4-7). One explanation for these findings is the weak affinity of loratadine for CNS receptors involved in sedation, including histamine H₁-receptors, α₁-adrenoceptors and acetylcholine receptors (5). In a recent study displacement of [³H]-mepyramine binding was studied in membranes from guinea-pig lung vs. cerebral cortex as a measure of affinity for peripheral vs. CNS histamine receptors. Loratadine was selective for lung vs. cortex, while other antihistamines, including terfenadine, astemizole, mequitazine and chlorpheniramine, were not selective. From these results it was concluded that the lack of significant sedative effects shown by loratadine was due to its poor

HUPERZINE A - AN INTERESTING ANTICHOLINESTERASE COMPOUND FROM THE CHINESE HERBAL MEDICINE

Jiří Patočka

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Summary: Huperzine A, alkaloid from the Chinese herbal medicine Qian Ceng Ta, which is prepared from the moss *Huperzia serrata*, has been used in China for centuries to treat fever and inflammation. Huperzine A is a strong inhibitor of cholinesterases with high selectivity to acetylcholinesterase and in China is developed as therapeutic against Alzheimer's disease. May be that huperzine A will be better than other centrally active anticholinesterases in treating this neurodegenerative disorder. Huperzine A appears to have additional pharmacological properties that make it an attractive candidate therapy for clinical trials.

Key words: Huperzine A; Alkaloid; Inhibitor of Acetylcholinesterase; Alzheimer's Disease; Treatment

Introduction

The alkaloid compound, huperzine A, was discovered in the Chinese herbal medicine called Qian Ceng Ta (14). This traditional remedy, which is prepared from the moss *Huperzia serrata*, has been used in China for centuries to treat fever and inflammation.

Chemistry

Huperzine A is an unsaturated sesquiterpenic compound with pyridone moiety and primary amino group (Fig. 1) $C_{15}H_{18}N_2O$. MW = 242.32. Chemically 9-amino-13-ethylidene-11-methyl-4-azatricyclo[7.3.1.0(3,8)]tridec-3(8),6,11-trien-5-one. Compound is optically active and in the moss is present only its (-)-enantiomer. The pyridone ring is planar and the stereochemistry of the C(11)-C(12) double bond is E. It is white solid soluble in aqueous acids and $CHCl_3$ (3).

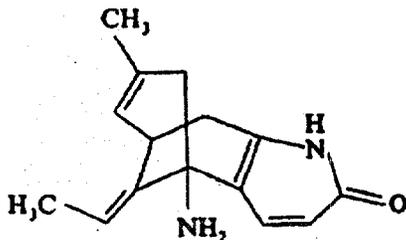


Fig. 1: Chemical structure of huperzine A

Biochemistry

Huperzine A is a potent reversible inhibitor of cholinesterases, i.e. acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) with on- and off-rates that depend on both the type and the source of enzyme. A low dissociation constants K_i was obtained for mammalian-AChE-huperzine A (2040 nM) compared to mammalian BuChE-huperzine A (20-40 μ M) (1). This indicates that the thermodynamic stability of huperzine-cholinesterase complex may depend on the number and type of aromatic amino acid residues in the catalytic pocket region of the enzyme molecule. The mechanism of the inhibition of acetylcholinesterase (AChE) is stereoselective. (-)-Huperzine A, which is in drug, was the more potent enantiomer with a K_i value of 8 nM. (+)-Huperzine A inhibited the enzyme 38-fold less potently with a K_i value of 300 nM. Racemic huperzine A was about two-fold less potent than the more active enantiomer. The mechanism of inhibition of rat cortical AChE for all three compounds was of the mixed linear competitive type (9). Very similar results were obtained with enzymes from other sources (13). The crystal structure of the complex of AChE with optically pure huperzine A at 2.5 Å resolution shows an unexpected orientation for the inhibitor with surprisingly few strong direct interactions with protein residues to explain its high affinity. An analysis of the affinities of structural analogues of huperzine A, correlated with their interactions with the protein, shows the importance of individual hydrophobic interactions between huperzine A and aromatic residues in the active-site gorge of AChE (12, 13). Based on docking studies and the pharmacologi-

cal results reported for huperzine A and its analogues, it was predicted that huperzine A binds to the bottom of the binding cavity of AChE with its ammonium group interacting with Trp84, Phe330 and Asp72 and to the opening of the gorge with its ammonium group partially interacting with Trp279. At the catalytic site, three partially overlapping subsites of huperzine A were identified which might provide a dynamic view of binding of huperzine A to the catalytic site (7, 10).

Neurochemistry

AChE was assessed in rats after acute and chronic administration of huperzine A. Forty-five min after a single injection of huperzine A (0.5 mg/kg, i.p.) the activity of AChE was significantly decreased by 15-30 per cent in hippocampus, striatum and septum. The activity of cholineacetyl transferase (ChAT) was not altered. In the hippocampus high affinity choline transport (HACT) was altered by 25 per cent, whereas no effect in the striatum was observed. After 90 min, both inhibition of AChE and attenuation of HACT had returned to control values. After 7 days chronic application of huperzine A (twice a day) at 0.5 mg/kg, the activity of AChE was significantly reduced by 20-30 per cent in every region of the brain studied. HACT in the hippocampus was reduced by 28 per cent, 45 min after the last injection, but in the striatum there was no effect. The activity of ChAT was not affected in any region of the brain studied (8).

Tang et al. (15) show that huperzine A can produce a long-term inhibition of AChE activity in the acetylcholine levels up to 40 per cent at 60 min. There is considerable regional variation in the degree of acetylcholine elevation after huperzine A with maximal values seen in frontal (125 per cent) and parietal (105 per cent) cortex and smaller increases (22-65 per cent) in other brain regions. A comparable effect was also observed in studies, in which, over a range of 0.1-2.0 mg/kg of huperzine A administered i.p., significantly inhibits of AChE activity in all brain region tested (hippocampus, hypothalamus, striatum and frontal cortex) and decreases level of brain acetylcholine (16).

Pharmacology

Huperzine A at concentrations 1 to 100 μ M does not significantly alter the electrically evoked release of 3 H-acetylcholine from cortical slices. With the exception of the highest concentrations (600 M) the displacement effect of huperzine A for cholinergic lipands is stronger for 3 H-nicotine than for 3 H-QNB. Autoradiographic study in the mouse shows that 60 min after i.v. injection (183 μ g/kg) huperzine A is particularly concentrated in certain areas such as frontoparietal cortex, nucleus accumbens, hippocampal, and striatal cortex. Radioactivity is practically absent in the whole body at 12 hr (15).

Huperzint A in doses from 0.4 to 0.5 mg/kg, i.p., significantly ameliorated the AF64A-induced memory deficit in rats in the radial maze. These results suggest that disrupting working memory induced by cholinotoxine AF64A can be effectively ameliorated by huperzint A (18). Very similar effects were obtained with huperzine A in doses from 0.1 to 0.4 mg/kg, p.o., on memory impairments induced by scopolamine. The comparison with other AChE inhibitors shows that huperzine A is the most selective AChE inhibitor, and improved the working memory deficit significantly better than did tacrine or donepezil (2). The results with natural (-)-huperzine A and synthetic (+/-)-huperzine A indicate a similar biological effects, but the racemic mixture of (+/-)-huperzine A has a weaker biological activity than the natural product (6).

Huperzine A in dose 0.1 mg/kg, in conscious rabbits produced, already 30 sec after i.v. administration, an alert EEG pattern, which showed decreases of lower frequency components and the total EEG power in cortical area, and the dominant frequency transferred from delta rhythm to theta rhythm in hippocampus and the same effects were observed with physostigmine in the dose of 0.1 mg/kg. Intravenously administered huperzine A in dose 0.2 mg/kg as well as physostigmine in dose 0.3 mg/kg antagonized the EEG effects of scopolamine (0.3 mg/kg i.v.). That results indicate that the effects of huperzine A are closely related to the action on the central cholinergic system (5).

Huperzint A appears to have additional pharmacological properties that make it an attractive candidate therapy for clinical trials. In studies using cell cultures from the hippocampus and cerebellum of rat embryos, have been shown that huperzine A decreases neuronal cell death caused by toxic level of glutamate (14). In addition to the loss of cholinergic function in patients with AD, glutamatergic and GABAergic neurotransmitter systems may also be compromised. Glutamate activates N-methyl-D-aspartate receptors and increases the flux of calcium ions into the neurons, which in sufficient concentration can kill the cells.

Huperzine A has been also testing as a prophylactic drug against soman and other nerve gas poisoning with very good effect (4).

Pharmacokinetics

Pharmacokinetic of huperzine A was studied in six volunteers after a single oral dose of 0.99 mg and drug concentrations were assayed by reverse phase HPLC from 0.5 to 10 hrs. The time course of plasma concentrations conformed to a one-compartment open model with a first order absorption with $T_{0.5ka} = 12.6$ min, $T_{0.5kz} = 288.5$ min, $T_{max} = 79.6$ min, $C_{max} = 8.4$ μ g/litre, $AUC = 4.1$ mg/litre.min. From this result is clear that huperzine A is absorbed rapidly, distributed widely in the body, and eliminated at a moderate rate (11).

Medical use

Huperzine A has similar action to the drugs currently approved to treat Alzheimer's disease - tacrine (Cognex) and donepezil (Aricept). i.e. inhibits brain AChE and blocks the breakdown of acetylcholine, a chemical messenger in the brain that is important to memory function (14, 19). Reports from China, where perhaps 100,000 people have used huperzine A, suggest that it is at least as safe as the two approved Alzheimer's drugs. Not all informations from China are available and trustworthy. It is evident that huperzine A in China was not only clinically tested, but this compound is used as remedy in the form of tablets in Alzheimer's disease (17). Nevertheless, huperzine A is probably still a long way to medical use in Europe (14).

The ability of huperzine A to decrease neuronal cell death caused by toxic level of glutamate may make this compound a potential drug for reducing neuronal injury from strokes, epilepsy, and other disorders.

Huperzine A is a candidate drug against organophosphate nerve agent toxicity for its long-lasting antidotal efficacy and low toxicity (4). Prophylactic study make this drug promising as a protective agent against chemical weapons.

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EFFICACY STUDIES

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Pharmacokinetics of tablet huperzine A in six volunteers¹

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AIM: To study pharmacokinetics of tablet huperzine A (Hup-A) in Chinese volunteers to help establishing its drug administration schedule. **METHODS:** For 6 volunteers after a single oral dose of 0.99 mg, drug concentrations in plasma were assayed by reverse phase high pressure liquid chromatography (HPLC) at 0.5, 0.75, 1.0, 1.25, 1.5, 2, 4, 6, 8, and 10 h. The pharmacokinetic parameters were calculated with a 3P87 program by computer. **RESULTS:** The time course of plasma concentrations conformed to a one-compartment open model with a first order absorption. The pharmacokinetic parameters were as follows: $T_{1/2\alpha} = 12.6$ min, $T_{1/2\beta} = 288.5$ min, $T_{max} = 79.6$ min, $C_{max} = 8.4 \mu\text{g L}^{-1}$, $AUC = 4.1 \text{ mg L}^{-1} \text{ min}$. **CONCLUSION:** Hup-A was absorbed rapidly, distributed widely in the body, and eliminated at a moderate rate.

KEY WORDS huperzine A; cholinesterase inhibitors; high pressure liquid chromatography; pharmacokinetics; phase I clinical trials

Huperzine A (Hup-A), a new alkaloid first isolated from Chinese herb *Huperzia serrata* (Thunb) Trev⁽¹⁾, exhibited a selective inhibition on acetylcholinesterase (AChE)⁽²⁾. It potentiated the skeletal muscle contraction and increased muscle tones⁽³⁾, and enhanced rodent learning and memory⁽⁴⁾. Clinically, Hup-A improved muscle weakness of myas-

thenia gravis⁽⁵⁾ and memory in patients with impaired memory or Alzheimer's disease⁽⁶⁾. The plasma level of Hup-A following *iv* or *ig* [³H]Hup-A 13.9 MBq kg^{-1} in rats declined in two phases, the distribution phase and the elimination phase, with half-lives of 6.6, 149 min (*iv*) and 10, 203 min (*ig*) respectively⁽⁷⁾. This paper was to study the pharmacokinetics of Hup-A in healthy volunteers to help establishing its drug administration schedule in clinic.

MATERIALS AND METHODS

Drug According to Chinese National Standard tablet Hup-A (batch No 940112) was prepared by the Institute of Materia Medica, Zhejiang Academy of Medical Sciences. The purity of Hup-A was 99.5%. Each tablet contains Hup-A 0.09 mg. (\pm minor Hup-A as internal standard was synthesized and presented kindly by Dr HE Xu-Chang, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, and 3 mg L^{-1} was used for experiment.

Subjects Six Chinese volunteers (M 3, F 3), aged 27 ± 6 a and weighing 58 ± 7 kg were all healthy, not in pregnant or menstruation. Each volunteer was told about the aim and process of the study. Agreements were obtained from them before study. Each subject was given a single oral dose of 0.99 mg Hup-A tablet at 8 am after an overnight fasting. Breakfast was served at 10 am. Blood (5 mL) was collected from an indwelling catheter in antecubital vein before and at 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 2, 4, 6, 8, and 10 h after *po*. Plasma (2 mL) was taken for HPLC. Pharmacokinetic parameters were obtained by first calculating the parameters from each person and then taking average of the 6 parameters, using a 3P87 program provided by Chinese Mathematic-Pharmacological Society on the computer.

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HPLC Shimadzu LC-GA liquid chromatography was connected to SPD-6A uv spectrophotometric detector (Shimadzu) and Rheodyn 7125 sampler, recorded on C-R3A integrator (Shimadzu). The column was a Spherisorb C18 (150 mm × 5 mm inner diameter; 5 μm particle size). The mobile phase was methanol: water (45:55, vol/vol), 1.0 mL min⁻¹ at 30 °C column oven. The column effluent was monitored at 313 nm.

Plasma sample Add (±)-dinor Hup-A 100 μL to plasma 2 mL, add Na₂CO₃-NaHCO₃ buffer 1 mL (using NaOH 1 mol L⁻¹ to adjust pH to 11.9). Then add chloroform 7.5 mL, shake 2 min, and centrifuge at 1000 × g for 10 min. The organic phase was blown to dryness by N₂ at 40 °C. Dissolve the residue with HPLC mobile phase 50 μL, and 20 μL was applied to HPLC. Hup-A peak and (±)-dinor Hup-A peak were separated clearly. The retention times (Rt) of (±)-dinor Hup-A and Hup-A were 3.5 and 8.3 min, respectively (Fig 1).

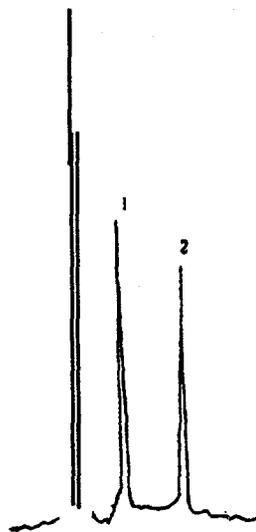


Fig 1. Chromatogram of blank plasma spiked with internal standard (peak 1, retention time 3.5 min) and Hup-A (peak 2, retention time 8.3 min).

Standard curve To the plasma containing (±)-dinor Hup-A add Hup-A 2.20, 4.43, 7.08, 8.85, and 17.70 μg L⁻¹, according to the ratio of Hup-A peak area to (±)-dinor Hup-A peak area in HPLC, a linear equation $\bar{Y} = 0.0188X - 0.0069$ was obtained ($r = 0.9988$). The minimal detect limit of plasma Hup-A

was 1.60 μg L⁻¹. The recovery of Hup-A was 95.7 ± 5.5% ($n = 9$) and coefficient of variation was 6.4%. According to measurements of 3 standard plasma Hup-A concentrations, intraday and interday variances were 5.5 × 7.4% ($n = 9$) and 6.0% - 9.9% ($n = 9$), respectively.

RESULTS

The plasma concentrations of Hup-A after oral administration of 0.99 mg within 10 h were fitted well to a one-compartment open model with a first-order absorption (Fig 2).

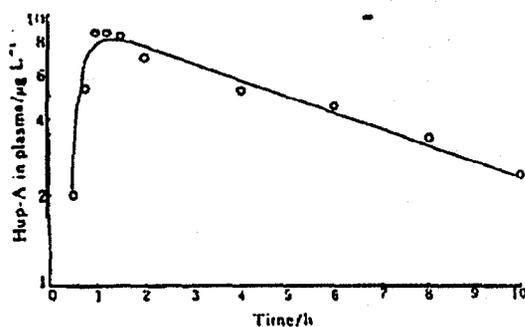


Fig 2. Mean plasma concentration-time curve after po tablet Hup-A 0.99 mg in 6 adults.

Hup-A was absorbed quickly after po with $T_{1/2}$ = 12.6 min and time peak for plasma averaged 79.6 min. It indicated that Hup-A was released and absorbed quite well *in vivo*. Plasma mean peak concentration after po was 8.4 μg L⁻¹, V_d/F was 0.108 L kg⁻¹, indicating that Hup-A was widely distributed *in vivo*. Mean elimination half life $T_{1/2}$ was 288.5 min, suggesting that Hup-A have a mild elimination rate (Tab 1).

DISCUSSION

Hup-A showed some advantages, compared with the first generation of ChE inhibitors such as physostigmine (Phy) and tetrahydroaminoacridine (THA), LD₅₀ value in mice for Hup-A ip was 1.8 mg kg⁻¹ and

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3), lthy, : was gree- Each up-A kfast ected efore i. 8, a for ed by and 3P87 tace

Tab 1. Pharmacokinetic parameters of Hup-A after po tablet 0.99 mg in 6 healthy volunteers. $\bar{x} \pm s$.

| Parameter | $\bar{x} \pm s$ |
|------------------------------|-----------------|
| K_e min ⁻¹ | 0.061 ± 0.017 |
| K_r min ⁻¹ | 0.0025 ± 0.0006 |
| $T_{1/2\alpha}$ min | 13 ± 5 |
| $T_{1/2\beta}$ min | 288 ± 63 |
| T_{max} min | 80 ± 9 |
| C_{max} μg L ⁻¹ | 8.4 ± 0.9 |
| T_{lag} min | 25.4 ± 1.8 |
| V_d/F L kg ⁻¹ | 0.108 ± 0.008 |
| AUC mg L ⁻¹ min | 4.1 ± 1.2 |

that for Phy was 0.6 mg kg⁻¹. Hup-A at optimal doses has a long term inhibition of AChE in rat brain (up to 360 min) and only 60 min for Phy⁽⁶⁾. The results of this paper showed that in human being $T_{1/2\beta}$ of Hup-A was 288.5 min. However, for bhy the $T_{1/2\beta}$ was 20 min⁽⁶⁾. Hup-A was absorbed rapidly, distributed widely in the body and eliminated at a middle rate⁽⁶⁾. Therefore it is better to take tablet Hup-A orally 2-3 times a day.

As a new ChE inhibitor, Hup-A shows some interesting cholinomimetic properties and its effects satisfy more closely established criteria for therapeutic use than effects of previously tested compounds. Hup-A is a new promising ChE inhibitor.

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石杉碱甲片在六名志愿者体内的药物动力学

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目的: 了解石杉碱甲片在人体内的药物动力学过程, 为设计临床用药方案提供依据。 **方法:** 用反相高效液相色谱法测定六名健康志愿者口服片剂0.99 mg后的血药浓度, 按3P87程序计算动力学参数。 **结果:** 石杉碱甲片在体内的药时过程符合一级吸收的一室开放模型。主要动力学参数: $T_{1/2\alpha}$, 12.6 min, $T_{1/2\beta}$, 288.5 min, T_{max} 79.6 min, C_{max} 8.4 μg L⁻¹, AUC 4.1 mg L⁻¹ min。 **结论:** 石杉碱甲吸收迅速, 属于中等速率消除类药物。

关键词 石杉碱甲; 胆碱酯酶抑制剂; 高压液相色谱法; 药物动力学; I期临床试验

ACUTE AND CHRONIC STUDIES WITH THE ANTICHOLINESTERASE HUPERZINE A: EFFECT ON CENTRAL NERVOUS SYSTEM CHOLINERGIC PARAMETERS

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Summary—High affinity choline transport, choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) were assessed in rats after acute and chronic administration of the AChE inhibitor Huperzine A. Acute treatment: Forty-five min after a single injection of Huperzine A (0.5 mg/kg i.p.) the activity of AChE was significantly decreased by 15–30% in hippocampus, striatum and septum. The activity of ChAT was not altered. In the hippocampus high affinity choline transport was attenuated by 15%, whereas no effect in the striatum was observed. After 90 min, both inhibition of AChE and attenuation of high affinity choline transport had returned to control values. A dose of 0.1 mg/kg (i.p.) did not produce significant effects. Similar results were obtained with physostigmine (0.25 mg/kg), although the duration of inhibition of AChE was shorter than that with Huperzine A.

Chronic treatment: After 3 days (twice a day), at 0.5 mg/kg, the activity of AChE was significantly reduced by 20–30% in every region of the brain studied. High affinity choline transport in the hippocampus was reduced by 25%, 45 min after the last injection, but in the striatum there was no effect. The activity of ChAT was not affected in any region of the brain studied. Thus, acute or chronic treatment with Huperzine A: did not alter ChAT; reduced high affinity choline transport in the hippocampus in a transient manner; and had a longer duration of action as an AChE inhibitor than physostigmine. Moreover, tolerance to low-toxicity doses of Huperzine A was minimal, contrary to what has been observed with other inhibitors of AChE.

Key words—chronic, Huperzine A, anticholinesterase, HACHT, ChAT, Alzheimer's disease.

The new cholinesterase (AChE) inhibitor Huperzine A (Fig. 1) is an alkaloid extracted from a *Lycopodium* found in China. It was reported to ameliorate learning and memory retention in rodents (Lu, Shou and Tang, 1988; Tang, Han, Chen and Zhu, 1986; Zhu and Tang, 1988). Moreover, improvements in memory, lasting for several hours after a single intramuscular injection, were reported in patients affected by impairment of memory or Alzheimer's disease (AD) (Zhang, 1986).

Recently, the acute action of Huperzine A was investigated in the CNS of the rat by Tang, De Sarro, Sugaya and Giacobini (1989), who showed a sustained increase in levels of acetylcholine (ACh) in brain of several hours duration. At the doses used, the inhibition of cholinesterase lasted three times longer than with physostigmine as well as producing significantly fewer side effects than physostigmine or tetrahydroaminoacridine (THA) (Tang et al., 1989).

However, the effect of Huperzine A on other central cholinergic parameters, such as the high affinity transport of choline and activity of choline acetyltransferase (ChAT) was not assessed *in vivo*. Neither was it determined if Huperzine A would be an effective cholinergic modulator during chronic

treatment. Here, it is reported that the inhibitory action of Huperzine A on AChE *in vivo* was effective at smaller doses than previously reported and, moreover, it persisted after chronic treatment, in all areas of the brain. Huperzine A also produced a transient inhibition of the high affinity transport of choline in the hippocampus.

METHODS

Animals

Male Sprague-Dawley rats (Zivic Miller Laboratories, Allison Park, Pennsylvania) were used. At the time of the experiment, the rats weighed between 275 and 350 g. For the duration of the experiment, the rats were housed in groups of 2 on a 12-hr light-dark cycle. Food and water were available *ad libitum*.

Administration of Huperzine A

Huperzine A and the reference inhibitor of AChE physostigmine salicylate were solubilized in saline and injected intraperitoneally (i.p.). The chronic treatment consisted of 9 injections, over a period of 4 days (twice a day, hence). The 9th and last injection was administered 45 min prior to sacrifice.

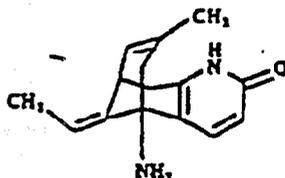


fig. 1. Molecular representation of Huperzine A.

Dissection of tissue

After decapitation, the brain was quickly removed and the various regions of the brain studied were dissected from each hemisphere, on a chilled metallic plate, according to Glowinski and Iversen (1966).

Activity of ChAT

Dissected areas of the brain were homogenized in 19 volumes of sodium phosphate buffer (75 mM, pH 7.4, 4°C) and the homogenate was frozen at -70°C, until subsequent analysis of enzyme. After thawing, homogenate (10 µl, 6 mg protein per ml) was added in duplicate to 10 µl of buffer-substrate mixture (McCaman and Hunt, 1965; Spyku, Goldberg and Sparber, 1972) comprising: sodium phosphate, 75 mM (pH 7.4); NaCl, 600 mM; MgCl₂, 40 mM; physostigmine, 2.0 mM; bovine serum albumin, 0.05%; choline (Ch) iodide, 10 mM and [³H]acetyl-coenzyme A, 0.57 mM. After 30 min of incubation at 37°C, the tubes were placed on ice and 150 µl of 3-heptanone, containing 75 mg/ml sodium tetraphenylboron, were added to each tube to extract the ACh (Fonnum, 1969). After vortexing, the samples were centrifuged and a 100 µl aliquot of the top (organic) layer was assayed for radioactivity, using liquid scintillation spectrometry.

Activity of acetylcholinesterase (AChE)

Dissected areas of the brain were homogenized in 19 volumes of sodium phosphate buffer (75 mM, pH 7.4, 4°C) and the homogenate was frozen at -70°C until subsequent analysis of enzyme. After thawing, the homogenate (10 µl, 6 mg protein per ml) was added in duplicate to 40 µl of buffer-substrate mixture, which contained: sodium phosphate (75 mM, pH 7.0, 4°C) and [³H]ACh iodide (10 mM). After 20 min of incubation at 37°C, the tubes were placed on ice and 150 µl of sodium tetraphenylboron/3-heptanone were added to each tube to separate ACh from the acetate (Fonnum, 1969). The samples were vortexed, centrifuged and placed at -70°C, until the bottom (aqueous) layer was frozen; the top (organic) layer was then removed by aspiration. Subsequently, the aqueous layer was thawed and a 25 µl aliquot was assayed for radioactivity, using liquid scintillation spectrometry.

High affinity transport of choline

Dissected areas of the brain were homogenized in 19 volumes of sucrose (0.32 M, 4°C) and centrifuged

(1000 g, 10 min, 4°C). The supernatant was then recentrifuged (20,000 g, 20 min, 4°C) and the resultant pellet was resuspended in 19 volumes of sucrose (0.32 M, 4°C). Duplicate aliquots (50 µl) of the suspension were then added to 500 µl of buffer (pH 7.4) comprising: Ch, 1.0 µM; [³H]Ch, 0.28 µCi; NaCl, 125 mM; KCl, 9.6 mM; MgSO₄, 4.2 mM; CaCl₂, 1.4 mM; dextrose, 10.0 mM and Tris base, 40.0 mM. In Na⁺-free buffer, 252 mM sucrose was substituted for sodium. After 8 min of incubation at 30°C, 1 ml of buffer (4°C) were added to each sample and tissue was collected onto GF/F filters (Whatman), by vacuum filtration. After washing with 10 ml of cold buffer, the filters were placed in scintillation vials and were assayed for radioactivity by liquid scintillation spectrometry. The Na⁺-dependent high affinity transport of choline was defined as the amount of choline transported into tissue, in the presence of Na⁺, minus that accumulated in the absence of Na⁺ (Yamamura and Snyder, 1973). Protein was assayed according to Lowry, Rosebrough, Farr and Randall (1951).

Statistical analysis

Differences were compared by multiple analysis of variance and *post-hoc* analysis, using the SYSTAT Statistical System (Evanston, Illinois, U.S.A.).

RESULTS

Figure 2 illustrates the effects of a single injection of small doses of Huperzine A on the activity of AChE in various regions of the brain. The data indicate that the inhibition of esterase was dose- and time-dependent in hippocampus, striatum and septum. At 45 min after the injection, the dose of 0.1 mg/kg, (i.p.) induced a slight but non-significant reduction in specific activity of AChE. At 0.3 mg/kg (i.p.), the activity of AChE was more strongly reduced ($P < 0.01$, $\times 0.001$, < 0.005 in hippocampus, striatum and septum, respectively). At these small doses,

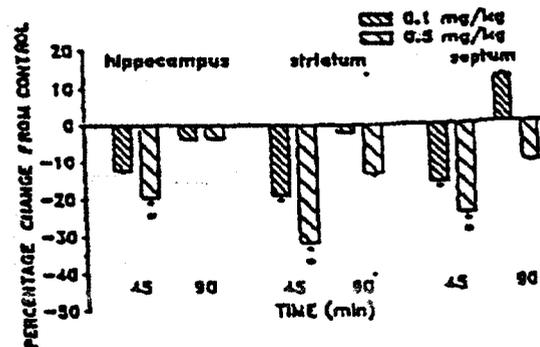


Fig. 2. Effect of acute injection of Huperzine A on activity of AChE in various regions of the brain. Values represent mean \pm SEM. Two-way ANOVA (repeated measures), $P < 0.001$. Multiple contrasts analysis for dose-effect at 45 min: 0.1 mg/kg, $P = 0.52$; 0.3 mg/kg, $*P < 0.01$. At 90 min: non-significant (N.S.). $N = 4-6$ rats/group.

the inhibition of AChE was mostly reversed by 90 min after the injection in all regions of the brain studied.

By comparison, a single injection of physostigmine (as the salicylate, 0.25 mg/kg i.p.) resulted in a more profound reduction in activity of AChE than char seen with Huperzine A (Fig. 2), ranging from 30 to 50% in parietal cortex, septum, hippocampus and striatum at 15 min after the injection (results not shown). However, the activity of AChE had reverted to control levels by 30 min after injection of physostigmine.

The specificity of Huperzine A on the metabolism of ACh was assessed by determining, in parallel, the activity of ChAT in each sample. The ACh-forming enzymatic activity was not influenced *in vivo* in the hippocampus or in the striatum by Huperzine A (results not shown). The specificity of Huperzine A on this cholinergic parameter, ChAT, was further compared to that of physostigmine (0.3 mg/kg i.p., 15 min after the injection) in cortex, septum, striatum and hippocampus. Physostigmine had essentially no effect on the activity of ChAT *in vivo* (results not shown).

As shown in Fig. 5, a single injection of Huperzine A produced a transient inhibition of the high affinity transport of choline in hippocampal synaptosomes. The transport activity was significantly ($P < 0.01$) reduced at 45 min. at the dose of 0.5 mg/kg (i.p.), whereas there was essentially no effect at 0.1 mg/kg (data not shown). By 90 min, the transport had returned to control values. High affinity transport of choline in the striatum was measured in parallel in the same animals at 45 and 90 min after the injection. The data in Fig. 3 show clearly that no inhibition of the uptake of choline took place at 0.5 mg/kg (i.p.), or at 0.1 mg/kg (i.p.) (data not shown).

The high affinity transport of choline was also assessed in various regions of the brain of rats injected with physostigmine (0.25 mg/kg i.p.). At

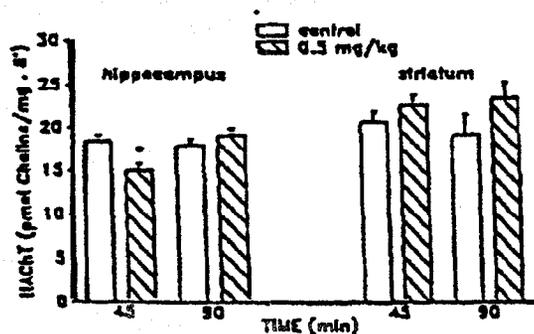


Fig. 3. Effect of acute injection of Huperzine A (0.5 mg/kg) on high affinity choline transport (HAChT) in hippocampus and striatum. Values represent mean \pm SEM. Two-way ANOVA (hippocampus), $P < 0.001$. Single contrast analysis for dose-effect: at 45 min, $*P < 0.01$; at 90 min, non-significant. Striatum: no significant differences. $N = 7-10$ rats/group.

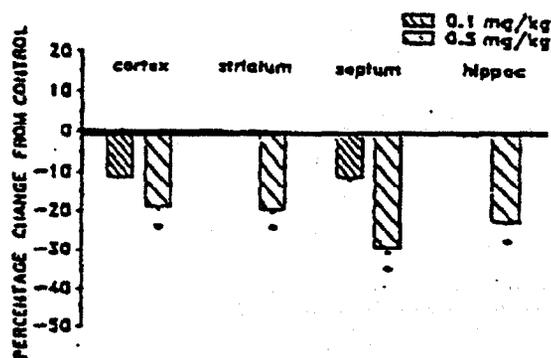


Fig. 4. Effect of 9 chronic injections of Huperzine A (4.3 days) on activity of AChE in various regions of the brain. Values represent mean \pm SEM. Two-way ANOVA (repeated measures), $P < 0.001$. Multiple contrasts analysis for dose-effect: at 0.1 mg/kg, N.S.; 0.5 mg/kg, $*P < 0.01$. $N = 6$ rats/group.

15 min, the transport was reduced significantly in hippocampus and parietal cortex by 34% and 37%, respectively, but not in the striatum (results not shown). By 30 min after the injection, the inhibition persisted significantly in the cortex and hippocampus.

The data in Figs 4 and 5 relate to the chronic treatment (twice a day for 4.3 days) with Huperzine A on the same parameters which were studied acutely. As shown in Fig. 4, the reduction in activity of AChE in the various regions of the brain, at the dose of 0.1 mg/kg (i.p.), did not reach significance. However, at 0.5 mg/kg, the results showed that activity of AChE was significantly reduced by 20-30% in every region of the brain studied.

The high affinity transport of choline was similarly influenced by chronic treatment with Huperzine A, as is shown in Fig. 5. The slight reduction in transport of choline in the hippocampus was not significant at

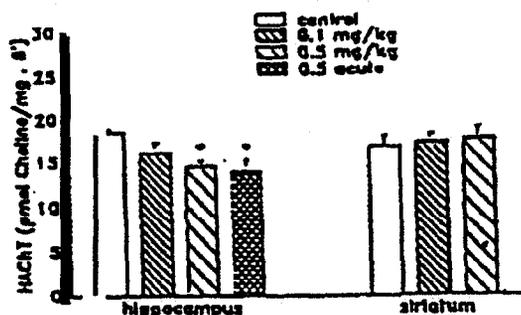


Fig. 5. Effect of 9 chronic injections of Huperzine A (4.3 days) on high affinity choline transport (HAChT) in hippocampus and striatum. Values represent mean \pm SEM. One-way ANOVA (hippocampus), $P < 0.005$. Single contrast analysis for dose-effect: at 0.1 mg/kg, N.S.; at 0.5 mg/kg, $*P < 0.01$. Striatum: no significant differences. The 0.5 mg/kg group (cross-hatch) was injected acutely and used as control. $N = 5-7$ rats/group.

0.1 mg/kg. However, at 0.5 mg/kg, high affinity transport of choline was reduced by 28% ($P < 0.01$), 45 min after the last injection of Huperzine A approximately to the same extent as in the acute controls included in this experiment. The high affinity transport of choline in striatal synaptosomes (Fig. 5), was not influenced by chronic treatment with Huperzine A, at either dose. The activity of ChAT was not affected *in vivo* in the hippocampus, striatum, cortex and septum after chronic treatment with Huperzine A (0.1 or 0.5 mg/kg i.p.) (results not shown).

Huperzine A-induced inhibition of the high affinity transport of choline was further investigated *in vitro*. Hippocampal synaptosomes were incubated with Huperzine A, at concentrations ranging from 10^{-10} M to 10^{-6} M, for periods of 5, 15 and 45 min. No consistent effect of the inhibitor of cholinesterase on high affinity transport of choline could be detected *in vitro*.

DISCUSSION

From the present data it is clear that Huperzine A-induced inhibition of AChE activity was as potent after chronic as it was after acute treatment. These results indicate that minimal tolerance to the drug occurred. This is important, since it is well established that tolerance develops to many of the effects of physostigmine (Costa, Schwab and Murphy, 1982; Genovese, Elmore and King, 1988). It has also been shown that the response to various inhibitors of AChE varies considerably after a second injection (360 min), especially in the case of THA (Hallak and Giacobini, 1989).

In their recent study with Huperzine A, Tang *et al.* (1989) used doses of 2 mg/kg (i.m.), with maximum inhibition of AChE occurring at 60 min and reported side effects, such as fasciculations. Inhibition of AChE was also studied at 30 min using smaller doses (ranging from 0.1 to 2 mg/kg i.p.) and maximum inhibition of AChE with minimal side effects occurred between 0.50 and 1 mg/kg (i.p.) (Tang *et al.*, 1989). In the present study, using two small doses of Huperzine A, administered intraperitoneally, at 45 min, it was observed that inhibition of AChE was not very effective at 0.1 mg/kg (i.p.). However, although inhibition of AChE attained 30–50% with physostigmine (0.25 mg/kg i.p.), as compared to 15–25% with Huperzine A (0.5 mg/kg i.p.) in various regions of the brain, it was observed that the duration of inhibition of AChE was longer than that with physostigmine. These results agree with previous findings (Tang *et al.*, 1989). Furthermore, at the small dose of 0.5 mg/kg (i.p.), no mortality or any side effects were observed, even after chronic treatment.

The action of Huperzine A on the activity of ChAT was also investigated *in vivo*. Acute or chronic treat-

ment with Huperzine A did not alter the activity of ChAT in any region of the brain studied. This finding complements the study of Hallak and Giacobini (1989), who reported no effect of various inhibitors of AChE *in vitro* (other than Huperzine A) on purified ChAT. Therefore, the reported *in vivo* increase in levels of ACh by Huperzine A (Tang *et al.*, 1989) was likely not to be mediated through an increase in the rate of synthesis of ACh.

In the same study, Tang and his coinvestigators showed that electrically-evoked release of ACh was not influenced by Huperzine A in slices of hippocampus. Neither was the release of ACh influenced by physostigmine, unless large concentrations were used (Hallak and Giacobini, 1989). Thus, it appears that release of ACh *in vivo* also may not be influenced by Huperzine A.

Another important effector of metabolism of ACh is the high affinity transport of choline (Tudek, 1985). According to the present studies, acute or chronic administration of Huperzine A was a potent inhibitor of high affinity transport of choline in the hippocampus *in vivo*. Physostigmine (Arweh, Simon and Kuhar, 1975; Sherman and Messamore, 1988) and THA (Sherman and Messamore, 1988) were also found to have a similar effect on transport of choline *in vivo*. However, in those studies, large doses of inhibitors of AChE, often accompanied by toxic effects, were used. Arweh *et al.* (1975) clearly showed that drugs affecting the turnover of ACh *in vivo* influenced the high affinity transport of choline, accordingly. For instance, physostigmine was shown to reduce turnover of ACh (Saelens, Simke, Schuman and Allen, 1974; Trabucchi, Cheney, Hanin and Costa, 1975) and muscarinic agonists, which increase turnover of ACh, increased high affinity transport of choline (Arweh *et al.*, 1975). The effect of inhibition of AChE on uptake of choline is believed to be mediated through a regulatory pre-synaptic control of high affinity transport of choline in response to the increase in content of ACh following inhibition of esterases (Yamamura and Snyder, 1973; Jope, 1979; Tamaru and Roberts, 1988; Breer and Knipper, 1990). The present results support this contention, since the effect of Huperzine A was completely reversible with time (Fig. 2) and not mediated through a direct interaction with the transporter (results not shown). Physostigmine also did not show any direct effect *in vitro* on synaptosomes in brain (Yamamura and Snyder, 1973), contrary to neostigmine (Yamamura and Snyder, 1973; Simon, Mittag, and Kuhar, 1975). These results indicate that inhibition of AChE may influence the high affinity transport of choline through a feedback-type regulation, rather than by operating directly on the transporter.

Hallak and Giacobini (1987) have hypothesized that *in vivo* treatment with an inhibitor of AChE which would not decrease turnover of ACh, would maintain long-lasting levels of the neurotransmitter

in the brain". Such may indeed be the case with Huperzine A. Although the present results could be interpreted as an indication that Huperzine A operates in the CNS according to the same mechanisms as those postulated for physostigmine, only the specific determination of the turnover of ACh will resolve the issue.

Another finding of this study that remains to be addressed is why the high affinity transport of choline was not decreased in the striatum, despite a potent reduction in the activity of AChE by both Huperzine A and physostigmine in this region of the brain. The striatum contains the greatest concentration of ACh in the brain (Sethy, Roth, Kuhar and Van Woert, 1973). Nevertheless, inhibition of AChE may not be accompanied by a significant elevation of ACh in striatum (Tang *et al.*, 1989). De Sarne, Pomponi, Giacobini, Tang and Williams (1989) have also shown that, after injection of a long-lasting derivative of physostigmine, increases in levels of ACh showed marked regional differences. Moreover, it has been appreciated for some time that regional variations exist among the effects of drugs on the high affinity transport of choline (Jope, 1979) and that the striatum often differs from other areas of the brain in its cholinergic responses to pharmacological challenges (Wecker and Deebarn, 1979; Sherman, Zigmond and Hanin, 1979).

In conclusion, it has been demonstrated that low-toxicity doses of Huperzine A could be used for several consecutive days and still exhibit full patency; hence, tolerance to Huperzine A, if it occurred, was minimal. Furthermore, the differences that have been shown in inhibition of AChE induced by Huperzine A and physostigmine are further indications that Huperzine A may be more effective and less toxic than physostigmine when a long term inhibition of AChE is required, e.g. in clinical treatment of diseases manifesting a cholinergic hypofunction.

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