Pharmacology Biochemistry & Behavior, Vol. 38, pp. 921–925. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

BRIEF COMMUNICATION

Nicotine Improves Cognitive Disturbance in Rodents Fed With a Choline-Deficient Diet

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Received 9 April 1990

SASAKI, H., M. YANAI, K. MEGURO, K. SEKIZAWA, Y. IKARASHI, Y. MARUYAMA, M. YAMAMOTO, Y. MATSUZAKI AND T. TAKISHIMA. *Nicotine improves cognitive disturbance in rodents fed with a choline-deficient diet.* PHARMACOL BIOCHEM BEHAV **38**(4) 921–925, 1991.—The effect of nicotine on learning disturbances was tested in rats. Rats were fed either a choline-enriched or a choline-deficient diet. Concentration of acetylcholine in the whole brain was significantly lower in rats fed with choline-deficient diet than rats fed with choline-enriched diet. Passive avoidance learning shows that rats on a choline-deficient diet showed significantly impaired learning compared to rats on a choline-enriched diet. Nicotine (0.04 mg/kg) administered intraperitoneally significantly potentiated learning in rats on a choline-deficient diet, as well as in rats on a choline-enriched diet. We, therefore, suggest that nicotine may potentiate learning in an acetylcholine-deprived brain.

Nicotine Tobacco Rat Acetylcholine Alzheimer's disease

EVIDENCE has been accumulating to show that cholinergic dysfunctions may play a particularly important role in the learning impairments that occur with old age. This evidence has stimulated interest in the possibility that these age-related impairments might be reduced by pharmacological manipulation of the cholinergic system. Dietary manipulation of choline, the precursor for the synthesis of acetylcholine, increases central cholinergic activity (9). After mice had been fed either free choline-deficient or choline-enriched diets, the retention of learning from a single-trial, passive avoidance task was superior in the choline-enriched old mice and inferior in the choline-deficient old mice (3).

As well, it has been demonstrated that there is a specific deficiency in acetylcholine, choline acetyltransferase, and acetylcholinesterase in autopsy material from patients with Alzheimer's disease (8). The severity of dementia is correlated to the neuropathologic indicators of cholinergic losses (19). The major anatomic lesion that correlates with Alzheimer's disease cholinergic deficiency is the loss of a majority of acetylcholine releasing neurons in the septal-diagonal band of the Broca-nucleus basalis system (7). Once these specific transmitter lesions were identified, the development of a treatment strategy for Alzheimer's disease became possible. If acetylcholine repletion could be accomplished pharmacologically, just as dopamine supplementation has been accomplished within the basal ganglia by the administration of L-dopa and carbidopa in Parkinson's disease, Alzheimer's disease might be similarly palliated (2). In 1986, the results of a study on the effects of one year average use of oral tetrahydroaminoacridine (a potent centrally acting anticholinesterase used for the treatment of memory deficiency in patients with Alzheimer's disease) were reported (23).

Nicotine, a major compound in tobacco smoke, stimulates the release of acetylcholine, norepinephrine, and dopamine from brain tissue (13). Administration of 0.2 mg/kg nicotine significantly improved the performance of passive avoidance retention in normal animals (5). Therefore, we assumed that nicotine would enhance the release of acetylcholine in acetylcholine-depleted conditions, and would improve learning. We adopted animal models with dietary choline deprivation (4) and tested whether nicotine improves the learning of these animals or not. The loss of acetylcholine in the brain is a phenomenon similar to Alzhei-

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mer's disease. Taking the world-wide popularity of smoking into account, it is worthwhile to test nicotine as a possible drug to combat Alzheimer's disease.

METHOD

Animals

Studies were carried out using 4-week-old male Wistar rats in cages under 12-h light-dark conditions. The rats were given free access to water, as well as to purified diets that were either choline-deficient or choline-enriched. Acetylcholine deprivation in rats on choline-deficient diets has been reported previously (18). The choline-enriched and deficient diets were purchased from Oriental-Kobo-Kogyo, Inc. (Tokyo). The choline-enriched diet contains approximately 4 mg/g and the deficient diet 0 mg/g of choline chloride. All of the remaining ingredients were the same as the standard rat chow containing approximately 1.6 mg/g of choline. The rats were divided into two groups, and were fed with choline-deficient (n = 34) and choline-enriched (n = 36) diets. When the rats became 14 weeks old (after 10 weeks on the diet), they were trained and tested using the passive avoidance test for cognitive responses. All experiments were performed between 8.00 a.m. and 10.00 a.m.

Spontaneous Movement in Rats

The spontaneous movements of rats in groups of four were measured using an Animex counter (Animex III, Shimazu Co.) before and after the intraperitoneal administration of either nicotine or saline. The four groups consisted of five rats, all of which were different from those used for passive avoidance learning. These groups can be divided as: control rats injected with either nicotine 0.01 mg/kg (n = 5), 0.04 mg/kg (n = 5) and 0.10 mg/kg (n = 5) and saline (n = 5). Spontaneous movement was measured for 7 min before and for 7 min after intraperitoneal injection and was divided into one-min intervals. We adopted this optimal nicotine dose and this minimum time after nicotine injection to study passive avoidance learning producing a minimum effect on spontaneous movement.

Passive Avoidance Learning in Rats

Passive avoidance learning was carried out according to the step-through procedure (15). The apparatus consisted of two compartments, one illuminated [300 × 250 mm; light (60 W) with a height of 200 mm to top of chamber], the other dark (200 × 150 mm with a height of 200 mm to top of chamber). The compartments were separated by a guillotine door (70 × 100 mm). The rat was placed into the illuminated safe compartment and then through the door the rat could enter the dark compartment and stand on a grid floor. Once all four paws were on the grid, a scrambled constant current (0.3 mA) and constant voltage (50 V, 50 Hz) foot-shock were delivered to the floor grid for 3 s. The rat could escape from the shock only by stepping back into the safe illuminated side. Then, the rat was returned to its home cage. Although the rats quickly escaped the shock administered, we could not measure the shock duration. However, variability of shock duration seems not to be systematically related to the different diet groups. High nicotine doses depress spontaneous movement in rats and even threaten the rats' lives, while low nicotine doses may not influence passive avoidance learning. Therefore, the optimal dose of nicotine and the time have to be selected to maximize the effect of nicotine on passive avoidance learning. The optimal dose of nicotine was determined to 0.04 mg/kg and the optimal time of nicotine or saline injection was determined to be 2 min prior to trial as described in the Results section. We administered either optimal nicotine dose 0.04 ml/kg (n = 10) or saline (n = 10) into the peritoneal cavity in choline-enriched (control) diet rats, and the optimal nicotine dose 0.04 mg/kg (n = 10) or saline (n = 10) into choline-deficient rats 2 min before trial. Passive avoidance learning was repeated on the second, third and fourth days in the same way as in the first trial, and the response latency in entering the dark compartment was measured. Nicotine or saline was administered prior to each trial. Results were recorded on the average latency time of step-throughs for each experimental group of rats. The latency of rats which did not move into the dark compartment during the observation period was calculated to be 300 s. We did not perform learning on rats which showed a latency period of more than 300 s.

Determination of Brain Acetylcholine and Choline

Whole brain choline (Ch) and acetylcholine (Ach) were measured in both control (n = 6) and choline-deficient rats (n = 4), which were not used in passive avoidance learning or spontaneous movement. The rats were sacrificed by microwave irradiation (microwave device NJE 2603 10 kW, New Japan Radio, Tokyo) at 9.0 kW for 0.75–1.15 s, which raised the brain temperature to 95.0 ± 1.7°C (11,16). The brain was removed from the skull and homogenized with the mixture of 3 ml of 0.05 M perchloric acid ($HClO_4$) and 30 nmol/30 μl of EHC using an ultrasonic cell disruptor (model US-300T, Nissei, Tokyo). The homogenate was centrifuged at 10,000 × g at 4°C for 15 min. The supernatant was filtered through 0.45 μm millipore filter, and then 5 μm of the supernatant was injected into liquid chromatography with electrochemical detection (LCEC) system for the determination of Ach and Ch (12,20). For the determination of protein in brain tissue obtained by centrifugation as described above, a solution of 1 N NaOH was added to the pellet to obtain a final volume of 10 ml and homogenized. The suspension was transferred to another tube and diluted with fifty volumes of the same alkaline solution. The protein was spectrophotometrically quantitated by the method of Brandford (1) with BSA as standard. The LCEC system consisted of a LC100P pump (Yokogawa Co., Ltd., Tokyo), a LC100S injector with 20 μl sample loop (Yokogawa Co., Ltd.), a LC-4A amperometric detector with platinum electrode (Bioanalytical System, BAS, West Lafayette, IN), and a LC100W/F-PC work station (Yokogawa Co., Ltd.) for LC data processing. The analytical column was BAS Acetylcholine Separation Column (Fig. 1). A borate column was used as precolumn, and an immobilized column containing acetylcholinesterase (AChE) and choline oxidase (Ch-oxidase) was used as postcolumn. Analytical column temperature was set at 35°C (with a BAS Temperature Controller LC 22A). The mobile phase was 0.05 M phosphate buffer, pH 8.4 containing 1 mM ethylendiaminetetraacetic acid disodium salt ($EDTD_2Na$) and 0.4 mM sodium 1-octanesulfonate (SOS). The flow rate was set at 0.8 ml/min. The electrode potential was set at +0.5V against an Ag/AgCl reference electrode for the detection of hydrogen peroxide. The principle of the technique is based on the separation of Ach and Ch in the separation column, followed by their enzymatic conversion through postcolumn reaction with AChE and Ch-oxidase to hydrogen peroxide which is detectable electrochemically by a platinum electrode.

Reagents

Acetylcholine (ACh) iodide and choline (Ch) iodide were purchased from the Sigma Chemical Co. (St. Louis, MO). Ethylhomocholine (EHC) iodide as an internal standard (IS) was synthesized from dimethyl-ϵ-amiono-1-propanol (Sigma Chemical Co.) and

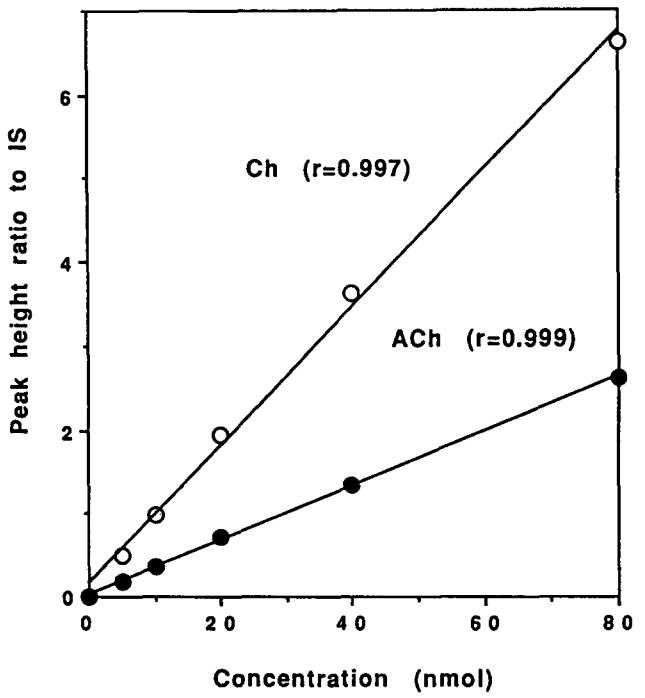

FIG. 1. Calibration lines for liquid chromatographic determination of choline (Ch) and acetylcholine (Ach). Ordinate shows peak height ratio to internal standard (IS) and abscissa shows concentration of Ch or Ach (n mol). (γ: correlation coefficient).

iodoethane (Sigma Chemical Co.) in the Department of Neuropsychopharmacology (Tsumura), Gunma University, School of Medicine. Other reagents for extraction and chromatography were of the highest available purity and purchased from commercial sources.

Statistics

Data are expressed as mean ± SD. Statistical analysis was performed using one-way analysis of variance and Duncan's multiple range test. Significance was accepted at $p<0.05$.

RESULTS

Spontaneous movements before and after saline or nicotine administration are shown in Table 1. Spontaneous movements before injection showed a tendency to decrease with time, were averaged during last 3 min and were 20 ± 12 counts/min (mean ± SD, n = 5), 20 ± 17 (n = 5), 19 ± 11 (n = 5) and 28 ± 13 (n = 5) in saline, nicotine 0.01 mg/kg, nicotine 0.04 mg/kg and nicotine 0.10 mg/kg, respectively. The spontaneous movements before injection were not significantly different among the four groups. We normalized spontaneous movement before injection as one hundred percent. Spontaneous movement tended to decrease after saline injection and there was significant difference between saline and nicotine 0.10 mg/kg one min after injection ($p<0.05$). Spontaneous movements after nicotine 0.10 mg/kg seem to be lower than the other three groups at 2 and 3 min although they were not significantly different. Nicotine 0.10 mg/kg sometimes cause instantaneous convulsions. Nicotine 0.04 mg/kg shows a slight decrease of spontaneous movement one min after injection but not after 2 min. Therefore, in order to minimize the effect of nicotine on spontaneous movement and to maximize the effect of nicotine on passive avoidance learning, we adopted nicotine 0.04 mg/kg administered 2 min prior to each trial in the following passive avoidance learning.

On the first day, there was no significant difference in latency times among the four groups. The latency of the rats' entrance into the dark compartment increased on subsequent test days in all groups (Fig. 2), except the latency time of the second day in the choline-deficient group administered with saline. From the second to the fourth day, the latency times in control rats administered with saline were higher than those of choline-deficient rats administered with saline ($p<0.01$). In both control and choline-deficient rats, nicotine increased latency times significantly as opposed to the latency times of those rats administered with saline ($p<0.01$). From the second to the fourth day, the latency times of control rats administered with nicotine- and choline-deficient rats administered with nicotine were not significantly different. On the third and fourth day, latency times were over 300 s in some rats in both control and choline-deficient rat administered with nicotine. Concentration of Ch and Ach in the whole brain

TABLE 1

SPONTANEOUS MOVEMENT BEFORE (CONTROL) AND AFTER SALINE OR NICOTINE

	Control (%)	Time After Injection (min) 1 (%)	2	3	4	5	6	7
Saline (n = 5)	100	90	85	60	80	40	60	50
		(35)	(95)	(65)	(55)	(30)	(40)	(80)
Nicotine								
0.01 mg/kg	100	110	85	63	50	20	17	60
(n = 5)		(60)	(50)	(45)	(10)	(25)	(10)	(45)
Nicotine								
0.04 mg/kg	100	67	110	40	36	30	28	25
(n = 5)		(63)	(68)	(20)	(26)	(5)	(10)	(15)
Nicotine								
0.10 mg/kg	100	35*	25	17	25	27	39	50
(n = 5)		(25)	(4)	(28)	(25)	(25)	(25)	(17)

Data of control movement was standardized as one hundred percent in each rat.
Data show mean ± (SD). *Shows significant difference from saline group ($p<0.05$).

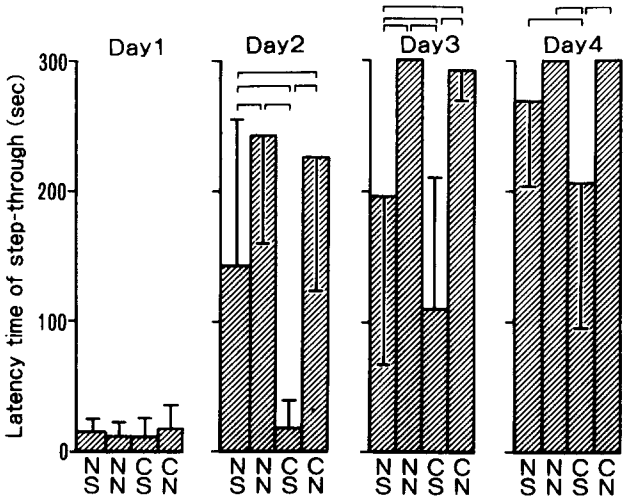

FIG. 2 Passive avoidance learning was performed upon rats fed on normal or choline-deficient diets that had received intraperitoneal administrations of either nicotine or saline, and had afterwards received an electric shock in the dark compartment. The latency times of step-through during passive avoidance learning from the first to fourth days in 24-h intervals are compared among the four groups: normal with saline (NS), normal with nicotine (NN), choline-deficient (rat) with saline (CS) and choline-deficient (rat) with nicotine (CN). Results were obtained using one-way analysis of variance and Duncan's multiple range test and are expressed by for $p<0.01$. Bars are mean ± SD.

tissue are shown in Table 2. There was significant difference of body weight ($p<0.05$). There was no significant difference of Ch between control and choline-deficient rats. There was significant difference of Ach between rats fed with choline-enriched and choline-deficient diet.

TABLE 2

CONCENTRATIONS OF CHOLINE (Ch) AND ACETYLCHOLINE (Ach) IN RAT WHOLE BRAIN

	Control (n = 6)	Ch Free (n = 4)
Body weight (g)	235 ± 13	200 ± 39*
Brain weight (g)	1.753 ± 0.097	1.689 ± 0.062
Protein (mg/g tissue weight)	99.7 ± 3.0	98.4 ± 11.6
Ch (pmol/mg protein)	189.0 ± 12.8	149.7 ± 37.2
Ach (pmol/mg protein)	298.0 ± 13.3	240.4 ± 26.2†

Each value is expressed as mean ± SD. *$p<0.05$ and †$p<0.01$.

DISCUSSION

Choline-deficient rats showed significantly lower latency times from the second to fourth days. The present choline-deficient model confirmed the previous results (3) that learning was superior in the choline-enriched mice and inferior in the choline-deficient mice. By using choline-deficient rats, we found that nicotine accelerated passive avoidance learning.

The effect of a choline-deficient diet on the concentration of acetylcholine in the brain tissue has been reported previously (6, 18, 20). Acetylcholine concentrations in whole rat brain vary with dietary choline consumption (18). We confirmed that acetylcholine in the whole brain is significantly lower in the rats with choline-deficient diet than the rats with choline enriched.

Nicotine is one of the major compounds found in tobacco smoke and it is widely used throughout the world. The effect of tobacco smoke on dementia has been debated. A study of epidemiologic aspects revealed that tobacco smoke had no significant effect on Alzheimer's disease (10). Tobacco smoke causes cerebral vascular diseases, thereby possibly resulting in the counterbalance of the effect of nicotine on learning. Nicotine has been known to facilitate learning and reduce aggression in normal rats (5,14). Acetylcholine deprivation in rats on choline-deficient diets has been reported previously (18,20). In the present study, we have suggested that nicotine facilitates learning in acetylcholine-deprived rats with learning disturbances. The effects of nicotine have been studied intensively, because nicotine is an addictive substance used in daily life. An epidemiological study suggested that Parkinson's disease was rarer among tobacco smokers (1). Intraperitoneal nicotine (0.4 mg/kg) administration improved movement in an animal model of Parkinson's disease (22). Nicotine stimulates the respiratory center in low doses and inhibits it in high doses (21). Similar biphasic nicotine stimulatory effects were observed in the airway nicotine receptors (17). Since the spontaneous movements were carried on a single administration of nicotine in small number of animals, these data may have little relevance to the performance of the animals in passive avoidance learning. However, in the present study, a high dose of nicotine seems to cause a reduction in spontaneous movement of rats at 1 min after injection (Table 1), and sometimes convulsions. Therefore, in the present study, we preferred to use relatively lower doses of nicotine to doses used in an animal model of Parkinson's disease (22). Nicotine reaches the brain within 10 s after administration and the half-time for elimination of nicotine is 30 to 60 min (13). We preferred to finish the passive avoidance tests within 10 min but never under 1 min.

The improvement of cognitive disturbance may be due to intense input of shock stimulation. We could not separate the learning improvement from increased shock sensitivity in the step-through procedure. Animal studies indicate that nicotine stimulates the release of norepinephrine and dopamine from the brain tissue and, depending on the dose, increases or inhibits the release of acetylcholine (13). If acetylcholine-deprived old rats and patients with Alzheimer's disease share a common factor resulting in the loss of learning ability (10), the fact that nicotine facilitates learning in acetylcholine deficient brain would be of great benefit in the future treatment of learning impaired patients.

ACKNOWLEDGEMENT

This study was supported by the Japan Smoking Research Foundation.

REFERENCES

1. Baron, J. A. Cigarette smoking and Parkinson's disease. Neurology 36:1490–1496; 1986.
2. Bartus, R. T.; Dean, R. L.; Beer, B.; Lippa, A. S. The cholinergic hypothesis in geriatric memory dysfunction. Science 217:408–417; 1982.
3. Bartus, R. T.; Dean, R. L.; Goas, J. A.; Lippa, A. S. Age-related changes in passive avoidance retention: modulation with dietary choline. Science 209:301–303; 1980.
4. Bradford, M. M. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principal of protein-dye binding. Anal. Biochem. 72:248–254; 1976.
5. Carson, P. S.; Silvette, H. Tobacco—experimental and clinical studies. Suppl. 3. Baltimore: The Williams & Wilkins Co.; 1975.
6. Cohen, E. L.; Wurtman, R. J. Brain acetylcholine: Control by di-

etary choline. Science 191:561–562; 1976.
7. Coyle, J. T.; Price, D. L.; Delong, M. R. Alzheimer's disease: a disorder of cortical cholinergic innervation. Science 219:1184–1190; 1983.
8. Davies, P.; Maloney, A. J. F. Selective loss of central cholinergic neurons in Alzheimer's disease. Lancet 2:1403; 1976.
9. Haubrich, D. R.; Wang, D. F. L.; Herman, R. L.; Clody, D. E. Increase in brain acetylcholine induced by choline or deanol. Life Sci. 17:975–980; 1975.
10. Heyman, A.; Wilkinson, W. E.; Stafford, J. A.; Helms, M. J.; Sigmon, A. H.; Weinberg, T. Alzheimer's disease: a study of epidemiologic aspects. Ann. Neurol. 15:335–341; 1984.
11. Ikarashi, Y.; Maruyama, Y.; Stavinoha, B. Study of the use of the microwave magnetic field for the rapid inactivation of brain enzyme. Jpn. J. Pharmacol. 35:371–387; 1984.
12. Ikarashi, Y.; Sasahara, T.; Maruyama, Y. Determination of choline and acetylcholine levels in rat brain regions by liquid chromatography with electrochemical detection. J. Chromatogr. 322:191–199; 1985.
13. Jaffe, J. H. Drug addiction and drug abuse. In: Gilman, A. G.; Goodman, L. S.; Gilman, A., eds. The pharmacological basis of therapeutics, 6th ed. New York: Macmillan Publishing Co.; 1980: 535–584.
14. Jaffe, J. H.; Jarvik, M. E. Tobacco use and tobacco use disorder. In: Lipton, M. A.; DiMascio, A.; Killam, K. F., eds. Psychopharmacology; A generation of progress. New York: Raven Press; 1978:1665–1676.
15. Jarvik, M. E.; Kopp, A. An improved one-trial passive avoidance learning situation. Psychol. Rep. 21:221–224; 1967.
16. Maruyama, Y.; Ikarashi, Y.; Blank, C. L. Evaluation of various microwave instruments for rapid inactivation of brain enzymes. Biogenic Amines 4:55–71; 1987.
17. Nakamura, M.; Haga, T.; Miyano, M.; Sasaki, H.; Takishima, T. Dose-response curves of central and peripheral airways to nicotine injections in dogs. J. Appl. Physiol. 61:1677–1685; 1986.
18. Orsetti, M.; Oggero, L. Behavioral and neurochemical modifications induced by dietary choline deprivation. Pharmacol. Res. Commun. 19:965–966; 1987.
19. Perry, E. K.; Tomlinson, B. E.; Blessed, G.; Bergmann, K.; Gibson, P. H.; Perry, R. H. Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. Br. Med. J. 2:1457–1459; 1978.
20. Potter, P. E.; Meek, J. L.; Neff, N. H. Acetylcholine and choline in neuronal tissue measured by HPLC with electrochemical detection. J. Neurochem. 41:188–194; 1983.
21. Sasaki, M.; Yamaya, M.; Hida, W.; Nakamura, M.; Sasaki, T.; Sasaki, H.; Takishima, T. Effect of hypercapnia on ventilatory response to intravenous nicotine administration in anesthetized dogs. Respir. Physiol. 78:177–186; 1989.
22. Sershen, H.; Hashim, A.; Lajtha, A. Behavioral and biochemical effects of nicotine in an MPTP-induced mouse model of Parkinson's disease. Pharmacol. Biochem. Behav. 28:299–303; 1987.
23. Summers, W. K.; Majovaski, L. V.; Marsh, G. M.; Tachiki, K.; Kling, A. Oral tetrahydroaminoacridine in long-term treatment of senile dementia, Alzheimer type. N. Engl. J. Med. 315:1241–1245; 1986.