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BRIEF COMMUNICATION

Nicotine Improves Cognitive Disturbance in Senescence-Accelerated Mice

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MEGURO, K., S. YAMAGUCHI, H. ARAI, T. NAKAGAWA, C. DOI, M. YAMADA, Y. IKARASHI, Y. MARUYAMA AND H. SASAKI. *Nicotine improves cognitive disturbance in senescence-accelerated mice.* PHARMACOL BIOCHEM BEHAV **49**(3) 769–772, 1994. —Senescence-accelerated mice (SAM), a murine model of age-related deterioration in learning ability, were studied as to the acetylcholine (ACh) contents in the brain tissues and the effect of nicotine administration. We found that the ACh content of SAM-P/8 (accelerated senescence-prone) mice was lower than that of SAM-R/1 (accelerated senescence-resistant) mice in the midbrain thalamus and the hypothalamus. In addition, an IP administration of nicotine was found to improve learning ability of SAM-P/8 as shown by performance of a passive avoidance task. Nicotine may potentiate cognitive function in SAM-P/8.

Nicotine Tobacco Acetylcholine Senescence-accelerated mice (SAM) Alzheimer's disease

RECENTLY, evidence has been accumulating that shows central cholinergic neuronal dysfunction may play an important role in learning impairments that occur with aging. These studies have stimulated interest in the possibility that such learning disabilities might be improved by pharmacological manipulation of the cholinergic neuron system. It has also been reported that there is a specific deficiency in central acetylcholine (ACh), choline acetyltransferase activity, and acetylcholinesterase activity in autopsy material from patients with Alzheimer's disease (5). The severity of dementia is correlated with neuropathologic indicators of cholinergic losses (4,17). Once this specific transmitter was identified, a treatment strategy for Alzheimer's disease became possible. If ACh repletion could be accomplished pharmacologically, just as dopamine supplementation has been accomplished within the basal ganglia by an administration of L-dopa and carbidopa in Parkinson's disease, so might Alzheimer's disease be similarly palliated (1).

Senescence-accelerated mice (SAM), which were established as a murine model of accelerated senescence by Takeda et al. (22), have been found to be valuable for studies of aging. It has been demonstrated that accelerated senescence-prone mice (SAM-P/8) show earlier onset and irreversible advancement of senescence following a normal process of development, as manifested by clinical signs and gross lesions such as alterations of general behavior, loss of skin glossiness, increased skin coarseness, hair loss, periophthalmic lesions, cataract, and increased lordokyphosis of the spine (9,22). In addition, spontaneous, age-related systemic amyloidosis accompanying the presence of the amyloid protein is observed in these mice (8). SAM-P/8 were observed to show age-related deterioration in learning abilities, that is, a significant deficit in a passive avoidance task and prolonged performance time in a multiple T-way maze task compared to control mice (senescence-resistant mice, SAM-R/1), the latter of which shows normal aging characteristics.

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It is known that nicotine stimulates the release of ACh, norepinephrine, and dopamine from the brain (12), and the administration of nicotine was found to improve the performance of passive avoidance retention in normal animals (3). Previously, we studied rats fed with a choline-deficient diet and found that nicotine improved learning ability (21). Therefore, we assumed that nicotine would enhance the release of ACh in acetylcholine-depleted conditions, and would improve learning. In this study, we investigated the effect of nicotine on learning ability in SAM-P/8.

METHOD

Animals

SAM-P/8 and SAM-R/1, donated by Prof. Takeda, Kyoto University, were reared in our laboratory under conventional conditions at 24 ± 2°C, fed on a commercial diet (CE-2, Nihon CLEA, Tokyo, Japan), and were given tap water ad lib. When mice became 12–16 months old, they were trained and tested using the passive avoidance test for cognitive responses. Old (12–16 months old) male SAM-P/8 (n = 33) and SAM-R/1 (n = 39) were used. All experiments were performed between 2000 and 2200 h, when the mice were awake and active.

Spontaneous Movement in SAM

The spontaneous movements of SAM in six groups were measured using an Animex counter (Animex III, Shimazu Co.) before and after the IP administration of either nicotine or saline. The six groups consisted of five mice, all of which were different from those used for passive avoidance learning. These groups were divided as: SAM-R/1 injected with nicotine 0.10 mg/kg (n = 5), 0.04 mg/kg (n = 5), 0.01 mg/kg (n = 5), and saline (n = 5), and SAM-P/8 injected with nicotine 0.04 mg/kg (n = 5) and saline (n = 5). Spontaneous movement was measured for 1 h in the activity box immediately after IP injection with either nicotine or saline.

Passive Avoidance Learning in SAM

Passive avoidance learning was carried out according to the step-through procedure (14). The apparatus consisted of two compartments, one illuminated [100 × 120 × 100 mm; light at the top of chamber (60 W)] and the other dark (100 × 170 × 100 mm). The compartments were separated by a guillotine door. The mice were placed in the illuminated safe compartment and then, through the door, the mice 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) at a constant voltage (50 V, 50 Hz) was delivered to the floor grid for 3 s. The mice could escape from the shock only by stepping back into the safe illuminated side. Then the mice were returned to their home cages. Although the mice quickly escaped the shock administered, we could not measure the shock duration. However, variability of shock duration does not seem to be systematically related to the different groups of SAM. High nicotine doses depress spontaneous movement in mice and even threaten their lives, whereas low nicotine doses may not influence passive avoidance learning. The optimal dose of nicotine was determined at 0.04 mg/kg as described in the Results section. We administered either nicotine 0.04 mg/kg (n = 10) or saline (n = 13) into the peritoneal cavity in SAM-P/8, and nicotine 0.04 mg/kg (n = 11) or saline (n = 8) into SAM-R/1 10 min prior to each trial of the passive avoidance task. Passive avoidance learning was repeated on the second and third days in the same way as in the first trial, and the response latency in entering the dark compartment was measured. Results were recorded on the average latency time of step-throughs for each experimental group of mice. The latency of mice that did not move into the dark compartment during the observation period was calculated to be 300 s. We did not perform the learning task with mice that showed a latency period more than 300 s, and such mice were considered to be "learned."

Determination of Brain ACh and Choline

Brain choline (Ch) and ACh were measured in SAM-P/8 (n = 6) and SAM-R/1 (n = 5) that were used in passive avoidance learning and spontaneous movement after saline injection. One week after the last learning, the mice were killed by microwave irradiation (microwave device NJE 2603 10kW, New Japan Radio, Tokyo, Japan) at 9.0 kW for 0.75–1.15 s, which raised the brain temperature to 95.0 ± 1.7°C (10,15). The brain was removed from the skull and dissected into seven regions according to the method of Glowinski and Iverson (6). The dissected tissue was homogenized with a mixture of 1 ml 0.05 M perchloric acid ($HCLO_4$) and 10 nmol/10 μl EHC using an ultrasonic cell disrupter (model US-300T, Nissei, Tokyo, Japan). The homogenate was centrifuged at 10,000 × g at 4°C for 15 min. The supernatant was filtered through a 0.45-μm millipore filter and then 5 μ supernatant was injected into a liquid chromatography with an electrochemical detection (LCEC) system for the determination of ACh and Ch (11,18). Tissue pellets obtained by centrifugation for the determination of protein were stored at −85°C until analysis. For assaying the protein concentration, a solution of 1 N NaOH was added to the pellets for the preparation of a final sample (10 ml) and homogenized. The homogenates obtained from tissues of the cerebellum, medulla-pons, hypothalamus, striatum, midbrain thalamus, hippocampus, and cortex were diluted with 1 N NaOH at the rate of 5-, 3-, 1-, 2-, 3-, 2-, and 10-fold, respectively. Using a Bio-Rad protein assay kit (Bio-Rad Labs., Richmond, CA), 0.1 ml of each of the above diluted homogenates was used for assaying protein concentration based upon the method of Bradford (2). Bovine serum albumin was used as the standard. The LCEC system consisted of an LC100P pump (Yokogawa Co., Ltd., Tokyo, Japan), an LC100S injector with 20-μl sample loop (Yokogawa), an LC-4A amperometric detector with platinum electrodes [Bioanalytical System (BAS), West Lafayette, IN], and an LC100W/F-PC work station (Yokogawa) for LC data processing. The analytical column was the BAS Acetylcholine Separation Column. A glassy carbon column was used as the precolumn, and an immobilized column containing AChE and Ch oxidase was used as the 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 nM $EDTD_2Na$ and 0.4 mM sodium 1-octanesulfonate (SOS). The flow rate was set at 0.8 ml/min. The electrode potential was set at +0.5 V against an Ag/AgCl reference electrode for the detection of hydrogen peroxide. The principle of the technique is based upon 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

ACh iodide and Ch iodide were purchased from the Sigma Chemical Co. (St. Louis, MO). Ethylhomocholine (EHC) iodide and an internal standard (IS) were synthesized from di-

methyl-3-amino-1-propanol (Sigma) and iodoethane (Sigma) in the Department of Neuropsychopharmacology (Tsumura), Gunma University, School of Medicine. Other reagents for extraction and chromatography were of the highest available purity and were purchased from commercial sources.

Statistics

Data are reported as means ± SD. Statistical analysis was performed by one-way analysis of variance (ANOVA) and Kruskal-Wallis rank test. Significance was accepted at $p < 0.05$.

RESULTS

Spontaneous movements of mice in SAM-R/1 injected with nicotine 0.10, 0.04, 0.01 mg/kg, and saline, and SAM-P/8 injected with nicotine 0.04 mg/kg and saline were 3821 ± 1232 (mean ± SD), 5506 ± 859, 5699 ± 678, 5874 ± 782, 4939 ± 911, and 5165 ± 960, respectively. Although there were not significant differences among the six groups, spontaneous movements after nicotine 0.10 mg/kg seem to be lower than the other five groups. Nicotine 0.10 mg/kg sometimes causes instantaneous convulsions. Therefore, 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 10 min prior to each trial in the following passive avoidance learning.

The latency times of the four groups are shown in Fig. 1, that is, P/8-S (SAM-P/8 treated with saline), P/8-N (SAM-P/8 treated with nicotine), R/1-S (SAM-R/1 treated with saline), and R/1-N (SAM-R/1 treated with nicotine). On the first day, there was no significant difference in the latency times among the four groups. As for SAM-R/1, the latency times of both groups of R/1-S and R/1-N increased on the second and third days without any significant difference. R/1 mice were considered to be "learned" on the third day because the latency times of all R/1 mice on the third day were over 300 s. After administration of nicotine, the latency time of P/8-N significantly increased when compared to that of P/8-S, both on the second day ($p < 0.01$) and on the third day ($p < 0.05$).

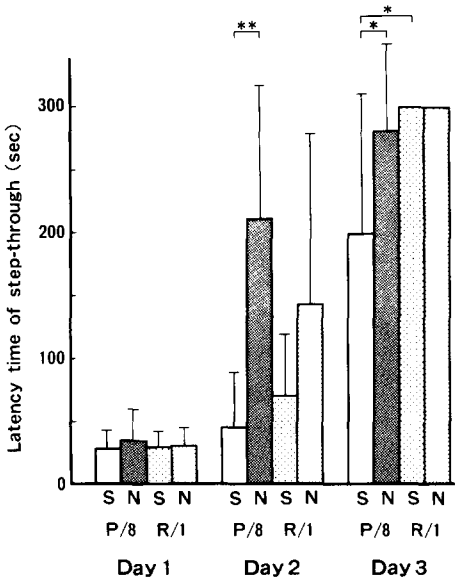

FIG. 1. Passive avoidance learning was performed on SAM-P/8 and SAM-R/1 that had received IP administrations of either nicotine or saline, and afterwards received an electric shock in the dark compartment. The latency times of step-through during passive avoidance learning from the first to the third days in 24-h intervals are compared among the four groups: SAM-P/8 with saline (P/8-S), SAM-P/8 with nicotine (P/8-N), SAM-R/1 with saline (R/1-S), and SAM-R/1 with nicotine (R/1-N). Statistical significance: $*p < 0.01$ and $**p < 0.05$.

Body weights in SAM-R/1 and SAM-P/8 were 33.1 ± 1.1 g and 27.8 ± 3.5, respectively. Body weights in SAM-R/1 were significantly larger than those in SAM-P/8 ($p < 0.01$). Whole brain weights of SAM-R/1 and SAM-P/8 were 400.5 ± 13.3 mg and 392.9 ± 10.6 mg, respectively. There were no significant differences. Tissue weights of brain regions were not significantly different between SAM-R/1 and SAM-P/8.

The regional brain ACh contents in SAM-P/8 and R/1 are shown in Table 1. The ACh contents in SAM-P/8 were lower than those of SAM-R/1 in almost all the regions, and statistical differences were found in the midbrain thalamus and the hypothalamus ($p < 0.05$).

DISCUSSION

SAM-P/8 showed significantly shorter latency times than SAM-R/1 on the third day. The present SAM-P/8 confirmed the previous results that learning was inferior in SAM-P/8 compared with SAM-R/1 (16,22). Previously, we observed that nicotine administered intraperitoneally significantly potentiated learning in rats on a choline-deficient diet (21). In the present study, by using SAM-P/8, we found that nicotine accelerated passive avoidance learning.

Nicotine is one of the major compounds found in tobacco smoke. Nicotine has been known to facilitate learning in normal rats (3,13). The effect of nicotine on learning disturbances was tested in rats fed with choline-deficient diets (21). Concentration of ACh in the whole brain was significantly lower in rats fed with choline-deficient diet than in 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. Because nicotine potentiated learning on rats on a choline-deficient diet, we suggested that nicotine may potentiate learning in an acetylcholine-deprived brain (21). In SAM-P/8, ACh showed a tendency to be lower in most of the regional brains and nicotine would stimulate the release of ACh and other substances from brain tissue (12). Nicotine also facilitated learning in SAM-R/1, but not significantly. Some SAM-R1 mice learned so quickly that the effect of nicotine may not be clear.

Nicotine doses were optimized so as to not disturb spontaneous movement and influence the learning of mice. We did not find significant differences in spontaneous movement among different doses of nicotine, but nicotine 0.10 mg/kg sometimes caused convulsions. Nicotine reaches the brain within 10 s after administration, and the half-time for elimination of nicotine is 30–60 min (12). Therefore, we preferred to use the same dose of nicotine and time of administration as was used in the rats (21).

The improvement of cognitive disturbance may be due to intense input of shock stimulation. Miyamoto et al. (16) studied differences in shock sensitivity using the flinch-jump threshold method. They observed no significant differences in shock sensitivity between SAM-P/8 and SAM-R/1. However, in the present study, we could not deny the learning improvement from increased shock sensitivity in the step-through procedure.

A study of epidemiologic aspects revealed that tobacco smoke had no significant effect on Alzheimer's disease (7). Animal studies indicate that nicotine stimulates the release of

TABLE 1

CEREBRO-REGIONAL CHOLINE (Ch) AND ACETYLCHOLINE (ACh) LEVELS IN SENESCENCE-ACCELERATED MICE (SAM), R/1 AND P/8

	Ch (pmol/mg protein)		ACh (pmol/mg protein)	
Brain Region	R/1 (n = 5)	P/8 (n = 6)	R/1 (n = 5)	P/8 (n = 6)
Cerebellum	252 ± 49	219 ± 115	123 ± 30	109 ± 16
Medulla-pons	453 ± 73	622 ± 378	356 ± 14	399 ± 104
Hypothalamus	196 ± 26	186 ± 66	385 ± 29	330 ± 25*
Striatum	176 ± 55	197 ± 71	544 ± 102	529 ± 61
Midbrain thalamus	190 ± 35	213 ± 78	351 ± 29	317 ± 27*
Hippocampus	87 ± 37	84 ± 44	257 ± 17	244 ± 15
Cortex	209 ± 40	219 ± 44	265 ± 30	249 ± 26
Whole brain	225 ± 30	242 ± 41	304 ± 17	284 ± 23

Data are represented as mean ± SD.
*Significantly different from control (R/1), $p < 0.05$ (Student's t-test).

ACh, norepinephrine, and dopamine from the brain tissue (12). Doses of nicotine produced a dose-related improvement in performance in the detection of signals in the rapid visual information processing task in patients with senile dementia of the Alzheimer type (19). If SAM-P/8 and patients with Alzheimer's disease share a common factor resulting in the loss of learning ability (7,14), the fact that nicotine facilitates learning in ACh-deficient brain would be of great benefit in the future treatment of learning-impaired patients.

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REFERENCES

1. Bartus, R. T.; Dean, R. L.; Beer, B.; Lippa, A. S. The cholinergic hypothesis in geriatric memory dysfunction. Science 217:408–417; 1982.
2. 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.
3. Carson, P. S.; Silvette, H. Tobacco-experimental and clinical studies. Suppl. 3. Baltimore: The Williams & Wilkins Co.; 1975.
4. Coyle, J. T.; Price, D. L.; Delong, M. R. Alzheimer's disease: A disorder of cortical cholinergic innervation. Science 219:1184–1190; 1983.
5. Davis, P.; Maloney, A. J. F. Selective loss of central cholinergic neurons in Alzheimer's disease. Lancet 2:1403; 1976.
6. Glowinski, J.; Iverson, L. L. Regional studies of catecholamines in the rat brain—1: The disposition of ^{3}H-norepinephrine, ^{3}H-dopamine and ^{3}H-dopa in various regions of the brain. J. Neurochem. 13:655–669; 1966.
7. 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.
8. Higuchi, K.; Matsumura, A.; Honma, A.; Takeshita, S.; Hashimoto, K.; Hosokawa, M.; Yasuhira, K.; Takeda, T. Systemic senile amyloid in senescence-accelerated mouse. A unique fibril protein demonstrated in tissues from various organs by unlabeled immunoperoxidase method. Lab. Invest. 48:231–240; 1983.
9. Hosokawa, M.; Kasai, R.; Higuchi, K.; Takeshita, S.; Shimizu, K.; Hamamoto, H.; Honma, A.; Irino, M.; Toda, K.; Matsumura, A.; Matushita, M.; Takeda, T. Grading score system: A method for evaluation of the degree of senescence accelerated mouse (SAM). Mech. Aging Dev. 26:91–102; 1984.
10. 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.
11. 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.
12. 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.
13. 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.
14. Jarvik, M. E.; Kopp, A. An improved one-trial passive avoidance learning situation. Psychol. Rep. 21:221–224; 1967.
15. Maruyama, Y.; Ikarashi, Y.; Blank, C. L. Evaluation of various microwave instruments for rapid inactivation of brain enzymes. Biogenic Amines 4:55–71; 1987.
16. Miyamoto, M.; Kiyota, Y.; Yamazaki, N.; Nagaoka, A.; Matsuo, T.; Nagata, Y.; Takeda, T. Age-related changes in learning and memory in the senescence-accelerated mouse (SAM). Physiol. Behav. 38:399–406; 1986.
17. 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.
18. Potter, P. E.; Meck, J. L.; Neff, N. H. Acetylcholine and choline in neuronal tissue measured by HPLC with electrochemical detection. J. Neurochem. 41:188–194; 1983.
19. Sahakian, B.; Janes, G.; Levy, R.; Gray, J.; Warburton, D. The effects of nicotine on attention, information processing, and short-term memory in patients with dementia of the Alzheimer type. Br. J. Psychiatry 154:797–800; 1989.
20. Sasaki, H.; Matsuzaki, Y.; Meguro, K.; Ikarashi, Y.; Maruyama, Y.; Yamaguchi, S.; Sekizawa, K. Vitamine B_{12} improves cognitive disturbance in residents fed with a choline-deficient diet. Pharmacol. Biochem. Behav. 43:635–639; 1992.
21. Sasaki, H.; Yanai, M.; Meguro, K.; Sekizawa, K.; Ikarashi, K.; Maruyama, Y.; Yamamoto, M.; Matsuzaki, Y.; Takishima, T. Nicotine improves cognitive disturbance in rodents fed with a choline-deficient diet. Pharmacol. Biochem. Behav. 38:921–925; 1991.
22. Takeda, T.; Hosokawa, M.; Takeshita, S.; Irino, M.; Higuchi, K.; Matsushita, T.; Tomita, Y.; Yasuhira, K.; Hamamoto, H.; Shimizu, K.; Ishii, M.; Yamamoto, T. A new murine model of accelerated senescence. Mech. Aging Dev. 17:183–194; 1981.