Counteraction of genipin to the trauma- and scopolamine-induced impairment of memory in mice

Kimiaki IMAKURA, Matsumi YAMAZAKI and Tetsuro MOHRI*

Department of Biodynamics, Hokuriku University School of Pharmacy

(*Received May 10, 1996. Accepted July 4, 1996.*)

Abstract

Administration (4 mg/kg, i.p.) of genipin, a component of the gardenia fruit, for 5 to 7 days was shown to counteract the impairment of learning and memory of mice after experimental head trauma and injection of scopolamine in assay methods of an active avoidance task, the latent learning test and spontaneous alternation behavior. These results suggest that genipin can be preventive against trauma-induced dementia and age-related memory impairment like senile dementia.

Key words genipin, memory impairment, active avoidance task, Y-maze, latent learning.

Abbreviations NGF, nerve growth factor ; FGF, fibroblast growth factor.

Introduction

Nerve growth factor (NGF) and fibroblast growth factor (FGF) have been known conspicuously to promote neurite outgrowth in cultured neuronal cells[1-3] and survival of the neuronal primary cultures of the brain tissues.[4,5] All of them have a regenerating effect on injured nerve tissues of the brain *in vivo*,[6-9] and infusion of them has been demonstrated to ameliorate the memory deficits induced by the brain lesions and aging in the rodents.[10-13]

Some herbal extracts and their components have been recognized to have the neuritogenic effect on the cultures of embryonal nervous tissues[14] and PC12h cells,[15] and some were effective on improvement of scopolamine-induced[16-18] and age-related[19] memory impairment in animals. Therefore effects on neuronal differentiation and survival in cultures seem to be a useful index to evaluate neurotrophic or nootropic activity of test drugs.

We previously demonstrated in PC12h cells that several iridoid compounds including geniposide and genipin had notable effects on the cells in induction of neurite outgrowth in parallel with increase in sensitivity to carbachol and membrane depolarization as determined by transition of the intracellular free calcium concentration.[20] We were then prompted to assess the promotive effect of the iridoid compounds on the learning and memory ability of experimental animals. We adopted in the present report three pharmacological methods to assess the learning- and memory-ameliorating effect of genipin on experimentally memory-deficient mice. Two of them, the latent learning and spontaneous alternation tests, are simple, but excellent, methods because the tests are performed in comparatively natural conditions, i.e. no use of intensively aversive reinforcement like starvation or electric shock in the training, to avoid possible emotional effect of test drugs on the strongly motivating condition itself such as fear or frustration. These two methods are important in testing the capacity of nonreinforced attention and learning[21] and rudimentary working memory,[22] respectively. On the other hand, the active avoidance task using the Skinner box tests the ability of mice learning to press the lever in an attempt to avoid electric shock. This method is very useful to evaluate memory of behavioral habituation in response to an aversive conditioning.

In this paper we will show in the three different

*〒920-11 金沢市金川町ホ3番地
北陸大学薬学部生物活性教室 毛利哲郎
Ho 3 Kanagawa-machi, Kanazawa, Ishikawa 920-11, Japan

和漢医薬学雑誌 13, 173－179, 1996

paradigms of memory assessment that administration of genipin to mice can ameliorate the memory impairment caused by a conventional head trauma or injection of scopolamine.

Materials and Methods

Chemicals : Genipin was purchased from Wako Pure Chemical Industries, Co., Osaka, Japan, and its purity was more than 98 %. It was dissolved in saline and checked for its stability in solution by silica gel TLC before use. It was repeatedly injected i.p. into animals at a daily dose of 4 mg/kg as specified in the results. Scopolamine hydrobromide was also purchased from Wako Pure Chemical Industries, Co. It was dissolved in saline just before use and injected i. p. into animals at a dose of 1 mg/kg 30 min before the start of specified tests.

Animals : Male mice of Std : ddY strain (Japan SLC, Shizuoka, Japan) weighing 25-30 g were used for the pharmacological experiments. They were kept in an air-conditioned room at 23±1°C and 55±5 % humidity with a 12-h light/12-h dark cycle and free access to food pellets and drinking water throughout experiments, unless otherwise mentioned. Cages each housing 5 mice were isolated by shielding from each other with cardboard in the breeding room during the experiment of the latent learning test.

Active avoidance task (Sidman) : Each mouse in the control and test groups (5 mice per group) were subjected to the test started by placing it in the Skinner box (15×17 cm and 17 cm high), which was equipped with a press lever attached to the wall and steel floor grids. An electric shock of current fixed throughout each session of the test in a range from 0.5-2 mA was repeatedly applied to mice through the grids, scrambled by an electric shock generator (MATYS MSG-001, Toyo Sangyo, Toyama, Japan) connected to the controller (MATYS MSG-002) and microcomputer, for 0.3 s usually with intervals of 5 s. When mice pressed the lever during the test period (30 min for every mouse in each session per day), the electric shock was temporarily delayed until 25 s after the lever pressing automatically. Numbers of the lever-pressing response (active avoidance of shock) and electric shock (reinforcement) applied were recorded for each mouse in every session in a series of test for 5 days.

Non-conditioned Y-maze task (spontaneous alternation behavior test) : The method is based principally on that of Sarter *et al.*[22] Y-maze box with three radially connected arms (each 40 cm long, 12 cm high and 3 cm wide in the floor) of V-shaped pathway was made of plywood, painted black inside. One mouse was placed in any of the closed ends of three arms at start and allowed to walk freely for exploration along the pathway of arms (entries) for 8 min. The arm entries (marked A, B and C) were sequentially recorded for that term and the percent alternation was calculated as the ratio of the number of consecutive entries into all three arms without repeat to that of total alternation opportunities (total entries minus 2) for each mouse.

Latent learning of water finding : The method is based on that described by Nabeshima *et al.*[21] An open field (50×30 cm and 15 cm high) of the apparatus made of plywood and painted gray inside was marked by thin black lines to divide the floor into 15 blocks (10×10 cm each). It had an alcove (10×10 cm and 10 cm high, open to the field in one side) with a drinking tube through the ceiling and water reservoir outside. For training the mouse not deprived of water, it was placed in the starting block far from the alcove and allowed to walk freely to explore in the field for 3 min. Mice that did not find the water tube in the alcove within the test period weve rejected in the following test trial. In the test trial 48 h after the training, each mouse, that had been restrained from drinking water for 20 h before the test trial, was placed again in the starting corner of the apparatus and allowed to explore in the field to find drinking water. Four parameters of latency in min, starting latency (time before movement to another from the starting block for exploration), entering latency (time before entering the alcove after start), finding latency (time before drinking water after entering the alcove) and drinking latency (time before drinking water after start) were recorded for each mouse. Exploration pathway in the test trial for each mouse to reach the water tube (ambulation) was evaluated by counting the blocks it passed. A part of the mice (genipin group) were injected with genipin for 7 consecutive

days, and the rest with vehicle, before the training. Each mouse in the genipin group and trauma control was given a blow to the head by dropping a 23 g metal weight from the height of 30 cm above head fixed on adequate paper cushion 24 h before the training trial. This produced a coma for about 10 s after the blow. The above two groups and control received the training before the test trial, while another group of mice (naive) did not receive the training.

Statistic analysis : All of the statistic analyses of the differences between average values of groups specified in the experimental results followed the method of ANOVA/Bonferroni's test.

Results

The increase in the number of the lever-pressing response in the active avoidance test with progress of the session was depressed by daily injection of scopolamine into mice 30 min before the start of test, and was significantly low compared with control (vehicle) at session 5 (Fig. 1). When genipin (4 mg/kg, i.p.) was administered to mice 1 h before start of the active avoidance test, i.e. 30 min before

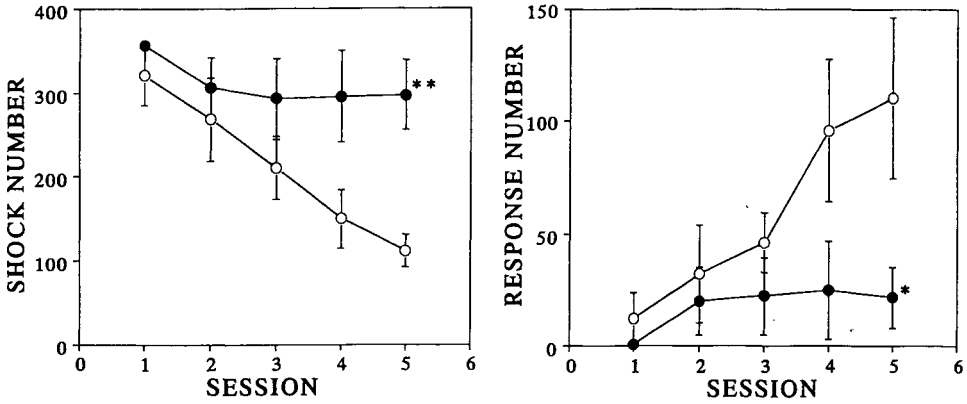

Fig. 1 Memory impairment by treatment of mice with scopolamine in the active avoidance test. Each group of mice was injected with scopolamine or saline (control) 30 min before the test of each session. The numbers of lever-pressing response and reinforcement (shock number) for each mouse in daily sessions shown in the abscissa were counted as described in Materials and Methods. (○) Control, (●) scopolamine. Each point shows the mean±S.E.M. $*p<0.05$, $**p<0.01$ vs. respective controls.

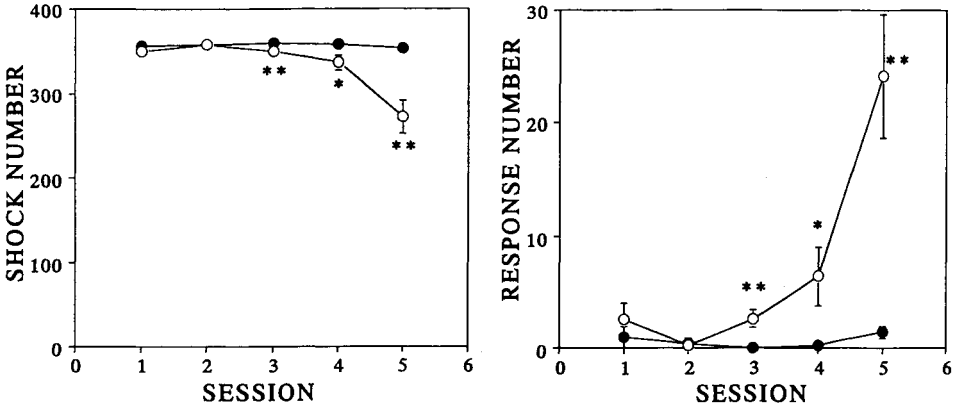

Fig. 2 Improvement of scopolamine-induced memory impairment by treatment of mice with genipin in the active avoidance test.
Each group of mice was injected with genipin or saline (control) 1 h before the test of each session. All of the mice were injected with scopolamine 30 min before the test. The test method was as described in the legend to Fig. 1. (●) Control, (○) genipin. Each point shows the mean±S.E.M (not shown in the values where each vertical bar is within the size of marks). $*p<0.05$, $**p<0.01$ vs. respective controls.

scopolamine (1 mg/kg, i.p.), every day, the average values of the lever pressing response increased exponentially with progress of the session, to the levels significantly high at session 3 and thereafter compared to the group of injection of scopolamine after injection of vehicle instead of genipin at respective sessions (Fig. 2). Reinforcement (number of electric shock) inversely decreased in the genipin group with progress of the session, to levels significantly lower than those in the control (vehicle) group at respective sessions 3-5. The treatment with genipin was without effect on normal mice until 5 days of administration at the same dose as above in the test (data not shown).

Administration of genipin (4 mg/kg per day, i.p.) for 7 days to mice before the test significantly increased the percent of spontaneous alternation in the Y-maze task after injection of scopolamine compared to the scopolamine control group (injected with scopolamine after 7 days of treatment with vehicle instead of genipin), recovering the percent alternation near the level of control (vehicle for both scopolamine and genipin) (Fig. 3). Total arm entries were not significantly affected by administration of either

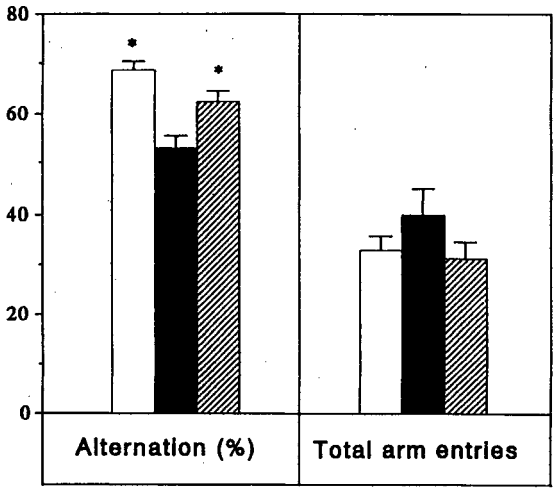

Fig. 3 Improvement of scopolamine-induced memory impairment by pretreatment of mice with genipin in the spontaneous alternation test with the Y-maze.
Saline (■, 8 mice) or genipin (9 mice, ▨) was injected for 7 days prior to the test and scopolamine into all of the mice 30 min before the test. Eight control mice (□) were injected with saline instead of genipin and scopolamine. Percent alternation and total arm entries were calculated for each mouse as described in Materials and Methods. Each value is the mean ± S.E.M. $*p < 0.01$ vs. saline-scopolamine.

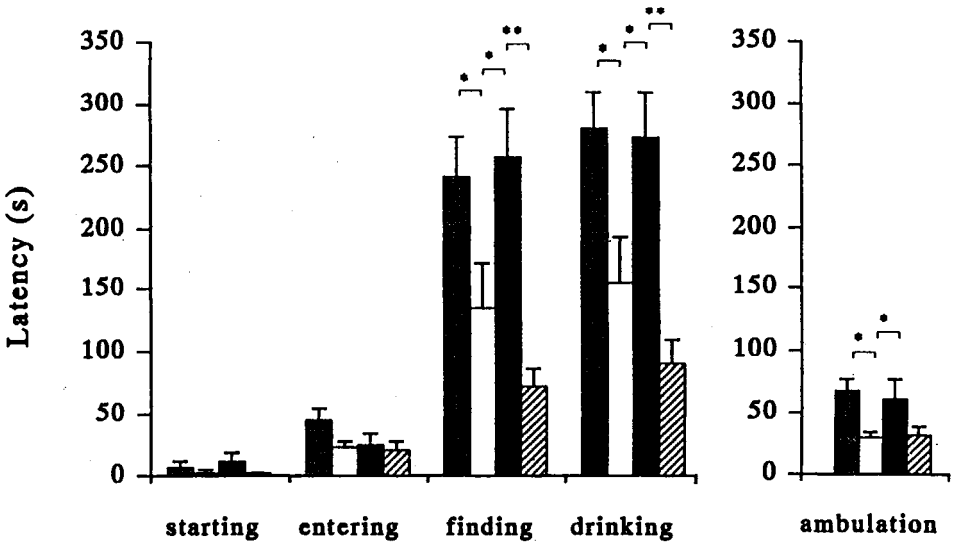

Fig. 4 Improvement of trauma-induced impairment of the latent learning by pretreatment of mice with genipin in the water finding test.
Saline (trauma control, ■, 9 mice) or genipin (▨, 7 mice) was injected for 7 days prior to trauma formation by the head blow. These two groups and control (saline for 7 days, no head blow, □, 10 mice) were subjected to the training and test trials as described in Materials and Methods. Another group of 10 mice (naive group, ▩) was subjected to the test trial without training. See Materials and Methods for definition of the parameters of latency and determination of ambulation. Each value is the mean±S.E.M. $*p<0.05$, $**p<0.01$.

Table I No effect of genipin and trauma on the exploratory behavior of mice in the training trial of the water finding test shown in Fig. 4.

	Group	Genipin dose (mg/kg)	N	Starting (s)	Entering (s)	No.of approaches to water tube (counts)	Ambulation (counts)
1	Naive	—	10	—	—	—	—
2	Control	—	10	7.7±3.2	79.5±14.4	5.3±1.0	52.7±10.8
3	Trauma control	—	9	9.8±3.1	66.1±8.8	3.6±0.9	74.7±14.2
4	Genipin	4	9	5.9±1.5	94.6±16.4	5.1±0.6	58.6±5.5

Each group (1-4) corresponds to that specified in Fig. 4. Starting and entering times and ambulation were determined within 3 min for each mouse in the same way as those of latencies and pathway to reach the water tube, respectively, in the test trial described in Materials and Methods. Each value is the mean±S.E.M.

genipin and scopolamine or vehicle and scopolamine.

Effect of genipin on the deficit of the latent learning was examined by the task of water finding. The average latency in finding and drinking water was significantly different between genipin and vehicle (trauma control) groups, showing shortening in genipin-injected mice (Fig. 4). The latency in finding and drinking of the genipin group was comparable to that of control, which had received neither injection of genipin nor trauma. The latency of trauma control, on the other hand, corresponded to that of the naive group in the both parameters. The latency in the other parameters was not significantly different between the groups. Ambulation was not found significantly to be different between the genipin and trauma control groups. Neither the administration of genipin nor trauma affected any of the parameters of the exploratory behavior of mice in the training trial, as compared between the three groups subjected to the training (Table I).

Discussion

Scopolamine is an antagonist of the acetylcholine receptor and proposed to inhibit acquisition of memory in the learning process. The short-term depression of memory by scopolamine is presumably due to its selective inhibition of the cholinergic neuronal transmission from the basal nucleus of forebrain to the hippocampus and cerebral cortex.[23] Central nervous system trauma is considered directly to destroy the local brain tissues and induce edema and the excitotoxicity of glutamate.[24]

Genipin, given to mice at daily dose of 4 mg/kg, i.p. for 5 or 7 days, showed counteraction to the memory deficit induced by head trauma or treatment with scopolamine in the three kinds of tests that are mutually complementary in assessment of different types of ability of learning or memory. These experimental results support a proposition that genipin could so effectively stimulate the cholinergic system as to counteract the memory impairment by scopolamine and trauma. The compound had no effect on the behavior of mice in the test dose as far as the ambulation or the number of total entries into arms is compared between control and test groups in the latent learning and Y-maze test, respectively.

Several iridoid compounds including genipin were found to be effective on protection of mice from acceleration of memory extinction and increase in failure of memory retrieval induced by repeated exposure to experimental stress at the daily dose of 50 mg/kg, p.o., in the passive avoidance task according to the step down method.[25] The present experiments have further elucidated that genipin can cause the avoidance of general deficit in ability of learning and memory after administration of scopolamine or brain injury due to trauma. Alzheimer's disease has a feature of shrinkage of the neuronal network in specific regions of the brain including the cerebral cortex and hippocampus in concomitant to dysfunction of cholinergic projections to the target tissues from the basal forebrain area. Therefore compounds like genipin, that counteracts the memory retardation

induced by scopolamine and head trauma, would reasonably be expected as preventive or therapeutic against senile and traumatic dementia.

The protein tyrosine kinase is implicated in the neuritogenic action of genipin[20] and also NGF[26,27] in PC12 cells. In memory formation and also induction of long term potentiation in the hippocampus, tyrosine kinase activity is considered to play an essential role.[28,29] Therefore the memory-ameliorating effect of NGF may be induced by its action on the tyrosine kinase activity of the central neurons, especially those in the basal forebrain (cf. Ref. No. 30). Based on these facts it is plausible that the promotive effect of genipin on memory in mice is dependent on the tyrosine kinase activity of cholinergic neurons in the central nervous system. Analysis of the effect of genipin on the signaling system involving tyrosine kinase(s) would be a point to solve the relation between the morphogenesis of neuronal cells and memory improvement in animals with it.

Acknowledgement

This work was supported in part by a grant from the special research fund of Hokuriku University (1995).

和文抄録

山梔子の一成分であるゲニピンを毎日4 mg/kg (i.p.), 5−7日間マウスに投与して，能動的条件回避試験 active avoidance test, 潜在学習試験 latent learning test, 及び自発的交代歩行試験 spontaneous alternation test により，頭部打撲，及びスコポラミン注射による実験的学習・記憶障害に対する効果を検定したところ，これら障害を有意に抑制することが示された。これらの結果は，ゲニピンが外傷性痴呆，及び老人性痴呆のような記憶障害を防御する可能性があることを示唆するものである。

References

1) Saito, H., Suda, K., Schwab, M. and Thoenen, H. : Potentiation of the NGF-mediated nerve fiber outgrowth by ginsenoside Rb_1 in organ cultures of chicken dorsal root ganglia. *Japan. J. Pharmacol.* **27**, 445-451, 1977.

2) Dichter, M.A., Tischler, A.B. and Greene, L.A. : Nerve growth factor-induced increase in electrical excitability and acetylcholine sensitivity of a rat pheochromocytoma cell line. *Nature* **268**, 501-504, 1977.

3) Togari, A., Baker, D., Dickens, G. and Guroff, G. : The neurite-promoting effect of fibroblast growth factor on PC12 cells. *Biochem. Biophys. Res. Commun.* **114**, 1189-1193, 1983.

4) Hartikka, J. and Hefti, F. : Development of septal cholinergic neurons in culture : Plating density and glial modulate effects of NGF on survival, fiber growth, and expression of transmitter-specific enzymes. *J. Neurosci.* **8**, 2967-2985, 1988.

5) Hatanaka, H., Nishio, C., Kushima, Y. and Tsukui, H. : Nerve-growth-factor-dependent and cell-density-independent survival of septal cholinergic neurons in culture from postnatal rats. *Neurosci. Res.* **8**, 69-82, 1990.

6) Kromer, L.F. : Nerve growth factor treatment after brain injury prevents neuronal death. *Science* **235**, 214-216, 1987.

7) Hefti, F. : Nerve growth factor (NGF) promotes survival of septal cholinergic neurons after fimbrial transection. *J. Neurosci.* **6**, 2155 2162, 1986.

8) Williams, L.R., Varon, S., Peterson, G.M., Wictorin, K., Fischer, W., Bjornklund, A. and Gage, F.H. : Continuous infusion of nerve growth factor prevents basal forebrain neuronal death after fimbria fornix transection. *Proc. Natl. Acad. Sci. U.S.* **83**, 9231-9235, 1986.

9) Abe, K., Ishiyama, J. and Saito, H. : Effects of epidermal growth factor and basic fibroblast growth factor on generation of long-term potentiation in the dentate gyrus of fimbria-fornix-lesioned rats. *Brain Research* **593**, 335-338, 1992.

10) Fischer, W., Sirevaag, A., Wiegand, S.J., Lindsay, R.M., and Bjorklund, A. : Reversal of spatial memory impairments in aged rats by nerve growth factor and neurotrophins 3 and 4/5 but not by brain-derived neurotrophic factor. *Proc. Natl. Acad. Sci. U.S. A.* **91**, 8607-8611, 1994.

11) Markowska, A.L., Koliatsos, V.E., Breckler, S.J., Price, D.L. and Olton, D.S. : Human nerve growth factor improves spatial memory in aged but not in young rats. *J. Neurosci.* **14**, 4815-4824, 1994.

12) Sinson, G., Voddi, M. and McIntosh, T.K. : Nerve growth factor administration attenuates cognitive but not neurobehavioral motor dysfunction or hippocampal cell loss following fluid-percussion brain injury in rats. *J. Neurochem.* **65**, 2209-2216, 1995.

13) Ishihara, A., Saito, H. and Nishiyama, N. : Basic fibroblast growth factor ameliorates learning deficits in basal forebrain-lesioned mice. *Japan. J. Pharmac.* **59**, 7-13, 1992.

14) Takemoto, Y., Ueyama, T., Saito, H., Horio, S., Sanada, S., Shoji, J., Yahara, S., Tanaka, O. and Shibata, S. : Potentiation of nerve growth factor-mediated nerve fiber production in organ cultures of chicken embryonic ganglia by ginseng saponins : Structure-activity relationship. *Chem. Pharm. Bull.* **32**, 3128-3133, 1984.

15) Mohri, T., Chiba, K., Yamazaki, M., Shimizu, M. and Morita, N. : Activation of PC12 cells by lipophilic components of *Panax ginseng*. *Planta Medica* **58**, 321-323, 1992.

16) Nitta, H., Matsumoto, K., Shimizu, M., Ni, X.-H. and Watanabe, H. : *Panax ginseng* extract improves the scopolamine-induced disruption of 8-arm radial maze performance in rats. *Biol. Pharm. Bull* **18**, 1439-1442, 1995.

17) Nishiyama, N., Wang, Y.-L. and Saito, H. : Beneficial effects of S-113m, a novel herbal prescription, on learning impairment model in mice. *Biol. Pharm. Bull.* **18**, 1498-1503, 1995.

18) Ni, X.-H., Otha, H., Watanabe, H. and Matsumoto, K. : *Panax ginseng* extract improves scopolamine-induced deficits in working memory performance in the T-maze delayed alternation task

in rats. *Phytother. Res.* **7**, 49-52, 1993.

19) Ohtha, H., Matsumoto, K., Shimizu, M. and Watanabe, H. : Paeoniflorin attenuates learning impairment of aged rats in operant brightness discrimination task. *Pharmacol. Biochem. Behavior* **49**, 213-217, 1994.

20) Yamazaki, M., Chiba, K. and Mohri, T. : Neuritogenic effect of natural iridoid compounds on PC12h cells and its possible relation to signaling protein kinases. *Biol. Pharm. Bull.* **19**, 791-795, 1996.

21) Nabeshima, T., Tohyama, K., Ichihara, K. and Kameyama, T. : Effects of benzodiazepines on passive avoidance response and latent learning in mice : Relationship to benzodiazepine receptors and the cholinergic neuronal system. *J.Pharmcol. Exp. Ther.* **255**, 789-794, 1990.

22) Sarter, M., Bodewitz, G. and Stephens, D.N. : Attenuation of scopolamine-induced impairment of spontaneous alternation behaviour by antagonist but not inverse agonist and agonist β-carbolines. *Psychopharmacol.* **94**, 491-495, 1988.

23) Alkon, D.L., Amaral, D.G., Bear, N.F., Black, J., Carew, T.J., Cohen, N.J., Disterhoft, J.F., Eichenbaum, H., Golski, S., Gorman, L.K., Lynch, G., McNaughton, B.L., Mishkin, M., Moyer Jr., J.R., Olds, J.L., Olton, D.S., Otto, T., Squire, L.R., Staubli, U., Thompson, L.T. and Wible, C. : Learning and memory. *Brain Research Reviews* **16**, 193-220, 1991.

24) Shohami, E., Cotev, S. and Shapira, Y. : A closed head injury model. In "Central Nervous System Trauma" (Ed. by Ohnishi, S. T. and Ohnishi, T.). CRC Press, New York, pp. 235-245, 1995.

25) Imai, T., Kishi, T., Inoue, H., Nishiyama, N. and Saito, H. : Effects of iridoids on sex- and learning-behaviors in chronic stressed mice. *Yakugaku Zasshi (Japan)* **108**, 572-585, 1988.

26) Inagaki, N., Thoenen, H. and Lindholm, D. : TrkA tyrosine residues involved in NGF-induced neurite outgrowth of PC12 cells. *Eur. J. Neurosci.* **7**, 1125-1133, 1995.

27) Fukuda, M., Gotoh, Y., Tachibana, T., Dell, K., Hattori, S., Yoneda, Y. and Nishida, E. : Induction of neurite outgrowth by MAP kinase in PC12 cells. *Oncogene* **11**, 239-244, 1995.

28) Abe, K. and Saito, H. : Tyrosine kinase inhibitors, herbimycin A and lavendustin A, block formation of long-term potentiation in the dentate gyrus in vivo. *Brain Research* **621**, 167-170, 1993.

29) Grant, S.G.N., O' Dell, T.J., Karl, K.A., Stein, P.L., Soriano, P. and Kandel, E.R. : Impaired long-term potentiation, spatial learning, and hippocampal development in *fyn* mutant mice. *Science* **258**, 1903-1910, 1992.

30) Koliatsos, V.E., Applegate, M.D., Knusel, B., Junard, E.O., Burton, L.E., Mobley, W.C., Hefti, F. and Price, D.L. : Recombinant human nerve growth factor prevents retrograde degeneration of axotomized basal forebrain cholinergic neurons in the rat. *Exp. Neurol.* 112, 161-173, 1991.