



Communications

Erinacine A increases catecholamine and nerve growth factor content in the central nervous system of rats

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Abstract

Nerve growth factor (NGF) is an essential protein for supporting growth and maintenance of peripheral sympathetic neurons. A novel diterpenoid erinacine, isolated from the cultured mycelia of *Hericium erinacium*, is known to have a potent stimulating effect on NGF synthesis. The effects of erinacine and related compounds in the brain *in vivo* are not known. In this study, we examined the effects of erinacine A on the production of NGF and catecholamines which stimulate NGF synthesis in the brain of rats. Rats were treated with erinacine A by intubation for the first 3 weeks from birth to weaning and intragastrically from weeks 4 to 5. Rats treated with this compound had increased levels of both noradrenaline and homovanillic acid in the locus coeruleus (LC) at 4 weeks of age and increased levels of NGF in both LC and hippocampus at 5 weeks of age. The effects of erinacine A were confirmed in the central nervous system in rats.

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1. Introduction

Nerve growth factor (NGF) is an essential protein for supporting the growth and maintenance of peripheral sympathetic neurons as well as facilitating the development of

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some sensory neurons for a brief period during early development [1]. NGF and its mRNA were barely detectable at birth, but their concentrations increased to peak levels at weaning [2].

In vivo NGF cannot penetrate the blood-brain barrier. Studies indicate that low-molecular-weight hericenones C-H from the fruiting bodies of *Herichium erinaceum* and erinacines A-I from the mycelia of the fungus stimulate NGF synthesis [3-8]. However, most of the experiments testing erinacines have been conducted in vitro, and few studies evaluated these compounds in the brain in vivo.

In this study, we examined the effects of erinacine A on the production of NGF in various brain regions of rats administered erinacine A. It has been demonstrated, using mice, that catecholamines stimulate NGF synthesis in an established fibroblast cell line, the L-M cell line [9]. Therefore, we also examined the effects of erinacine A on production of catecholamine in various brain regions both with and without the administration of erinacine A.

2. Methods and materials

Wistar strain female rats on day 14 of pregnancy were obtained from Japan SLC, Inc (Hamamatsu, Japan). Upon arrival, all rats were fed the 25% casein diet (AIN 76). The composition of the experimental diet is shown in Table 1. All rats, together with their pups, were maintained at 24°C with a 12-hour light/dark cycle and were provided free access to food and water. Twenty pups were divided into 2 groups (10 per group) immediately after birth; both groups of pups received an oral solution daily. The control group received 5% ethanol in a saline phosphate buffer (10 mL/kg body weight), and the treatment group received a solution of erinacine A (8 mg/kg body weight) dissolved in 5% ethanol and saline phosphate buffer. After weaning, all pups were fed the 25% casein diet, identical to that of their pregnant dams (Table 1). Two control groups, 4 and 5 weeks old, were given intragastrically (IG) a solution of 5% ethanol in a saline phosphate buffer (10 mL/kg body weight), and 2 erinacine A groups (identical age and sample size) were given IG the solution (8 mg/kg body weight). All IG administrations were done 5 times at 12-hour intervals.

After the last administration, the rats were decapitated in the anesthetized condition, and the various brain regions were weighed, frozen, and stored at -80°C until analyzed. The use

Table 1
Composition of the experimental diet

Ingredient	Diet (g/kg)
Casein	250
Corn starch	392
Sucrose	196
Cellulose	50
Corn oil	50
Mineral mixture ^a	50
Vitamin mixture ^b	10
Choline-Cl	1.5

All ingredients except for corn oil were purchased from Oriental Yeast Co, Ltd (Tokyo, Japan).

^a AIN-76 mineral mixture.

^b AIN-76 vitamin mixture.

of animals and the protocol were approved by the Animal Care and Use Committee of the University of Shizuoka.

The monoamine content was measured in the following brain regions of the rat at 4 weeks of age: the cerebral cortex, cerebellum, striatum, locus coeruleus (LC), hippocampus, amygdala, brain stem, and hypothalamus. Tissues were sonicated in 1 mL 0.1 N perchloric acid solution.

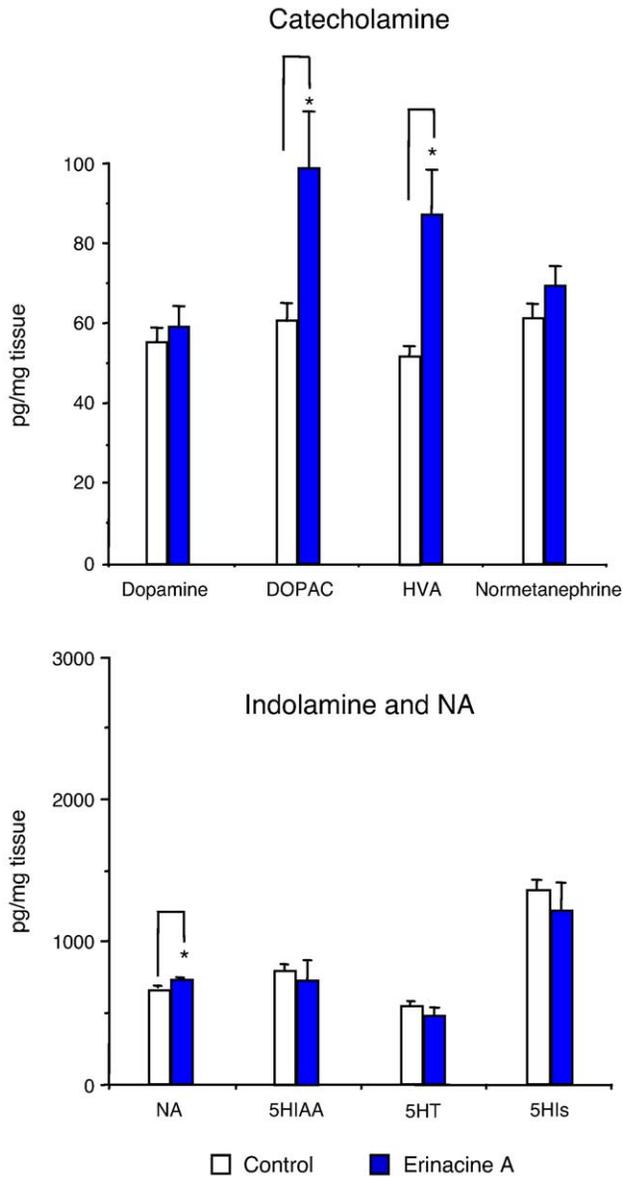


Fig. 1. Monoamine content in the locus coeruleus of rats fed the 25% casein diet simultaneously administered with (n = 5) or without erinacine A (n = 5). Values are mean ± SEM. *Significantly different from the control (P < .05).

After centrifugation at 15 000g for 10 minutes, the supernatant was filtered through a 0.45- μ m syringe filter (TOYO cellulose acetate; Toyo Roshi, Tokyo, Japan) and injected into a high-performance liquid chromatography for neurochemical analysis.

NGF content was measured in the following rat brain regions: olfactory bulb, LC, hippocampus, and the cerebral cortex, at 5 weeks of age. The tissues were homogenized with 19 mL/mg wet weight of homogenizing buffer (0.1 mol/L Tris-HCl, pH 7.6, containing 1 mol/L NaCl, 2 mmol/L EDTA, and 80 U/L aprotinin) with a sonicator at 4°C. The sonicates were centrifuged at 100 000g for 1 minute at 0°C, and their supernatants were used for NGF assay by enzyme immunoassay.

Data are presented as means \pm SEM. Statistical analysis was performed by Student *t* test. Differences between the 2 groups were considered significant at $P < .05$.

3. Results

The mean body weights of rat pups in the erinacine A and control groups at 4 weeks were 73.5 ± 1.6 and 71.9 ± 2.2 g, respectively. The mean total brain weights were 1.54 ± 0.02 and 1.57 ± 0.02 mg, respectively, for the same 2 groups. There was no significant difference in body and total brain weights between the 2 groups.

Monoamine content in the LC of rats fed the 25% casein diet, simultaneously administered with or without erinacine A, is shown in Fig. 1. In LC, 3-hydroxytyramine (dopamine) levels were unchanged, but its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), were significantly higher in the treated group than those of the control group. Noradrenaline (NA), which is mainly produced in the LC, was significantly

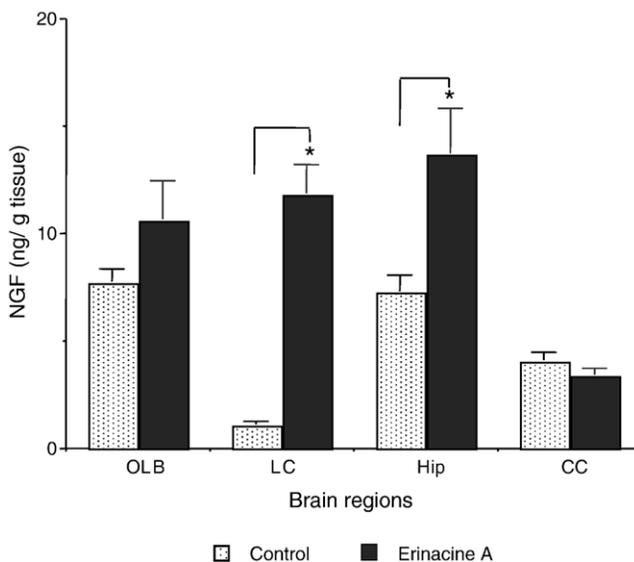


Fig. 2. NGF content in the brain of rats fed the 25% casein diet simultaneously administered with ($n = 5$) or without ($n = 5$) erinacine A. OLB indicates olfactory bulb; Hip, hippocampus; CC, cerebral cortex. Values are mean \pm SEM. *Significantly different from the control ($P < .05$).

different between the erinacine A and control groups. There was no significant difference in the amounts of 5-hydroxyindoleacetic acid (5-HIAA), 5-hydroxytryptamine (5-HT), and 5-HT + 5-HIAA (5-HIs) in the LC between the 2 groups (Fig. 1). For other brain regions, there was no significant difference in monoamine content between the 2 groups.

The mean body weights of rat pups in the erinacine A and control group at 5 weeks of age were 102.5 ± 2.7 and 99.6 ± 2.4 g, respectively. The mean total brain weights were 1.62 ± 0.03 and 1.60 ± 0.02 mg, respectively, for the same 2 groups. There was no significant difference in body and total brain weights between the 2 groups.

The effects of erinacine A on NGF in various brain regions in 5-week-old rats are shown in Fig. 2. The NGF content in the LC and hippocampus of the erinacine A-treated group was higher than that in the control group (Fig. 2). NGF content in the olfactory bulb and cerebral cortex was not significantly different between the control and erinacine A-treated group.

4. Discussion

In this experiment, rats treated with erinacine A from birth to weaning and also at 4 weeks of age had significantly different amounts of DOPAC, HVA, and NA in the LC, compared with the control group. At 5 weeks of age, the erinacine A group had significantly higher amounts of NGF in the LC and hippocampus, compared with the control group. Previous studies have reported that erinacine A increases NGF in astroglial cells [5]; however, these experiments were conducted in vitro. In our study, the effects of erinacine A on NGF content were conducted in vivo.

There are 2 possible explanations for the observations in studies using erinacine A. First, erinacine A stimulates production of NA enhancing NGF secretion in the LC and hippocampus. This is supported by research indicating that NGF mRNA is in the hippocampus, midbrain, and brain stem in adult rats [10]. NA has not yet been found to influence synthesis of neurotrophin, but a variety of observations predict that this neurotransmitter may well regulate the expression of NGF. For example, adrenergic receptors have been identified in astrocytes [11,12], and NA has been shown to regulate neurotrophin synthesis in whole brain and hippocampal astrocytes [13–15]. Because adrenergic receptors are also present in hippocampal neurons [16,17], it is possible that NA may similarly regulate neurotrophin synthesis in glial and neuronal cell populations in the hippocampus. One report suggests that the number of synapse connections in the hippocampus-septal system reaches adult level at weaning [17], and neurotrophins and neurotransmitters may collaborate to influence neuron and glial growth, survival, and function [18]. In this experiment, the LC-hippocampal system may be similarly regulated.

Second, it has been suggested that erinacine A increases the amounts of neurotrophin 3 (NT-3) in the LC and survival of noradrenergic neurons and also increases NA synthesis in the LC. NA in the LC stimulates NGF synthesis in the hippocampus. Experiments using cell culture models have revealed that although developing locus neurons were not influenced by NGF, they were responsive to neurotrophins NT-3 and NT4/5 [19]. Furthermore, high quantities of the enzyme tyrosine kinase C are found in the LC. Therefore, it is also expected that erinacine A stimulates NT-3. Moreover, NT-3 regulates NA transporter functions by creating an increase in NA transporter mRNA levels. NA transport regulates differentiation of noradrenergic neurons in the LC by promoting expression of tyrosine hydroxylase and dopamine β -hydroxylase. It has

also been reported that NT-3 elicited significant increases in survival of noradrenergic LC neurons [19]. NA stimulated NGF synthesis in the LC and hippocampus in our rat study.

In both explanations, it is suggested that the accumulation of NGF in the hippocampus would result in retrograde transport from LC as well as local synthesis. From our results, it is also intriguing to consider that erinacine A may affect neurotransmitter-neurotrophin collaborations, in particular, interactions of noradrenergic LC with the hippocampus [20,21], which degenerate in Alzheimer disease [22–24], suggesting that it may be regulated in a coordinated manner. However, further research is required for a more in-depth understanding of these results. There is no evidence that erinacine A is absorbed into the blood and crosses the blood-brain barrier or is localized in the brain tissue. To address this question, the concentration of erinacine A in the brain and blood must be measured.

In conclusion, erinacine A increased catecholamine and NGF content in the central nervous system of rats. Hence, it is a possible candidate for designed foods to stimulate NGF synthesis.

References

- [1] Hefri F, Hartikka J, Knusel B. Function of neurotrophic factors in the adult and aging brain and their possible use in the treatment of neurodegenerative diseases. *Neurobiol Aging* 1989;10:515–33.
- [2] Large TH, Bodary SC, Clegg DO, Weskamp G, Otten U, Reichardt LF. Nerve growth factor gene expression in the developing rat brain. *Science* 1987;234:352–5.
- [3] Kawagishi H, Ando M, Sakamoto H, Yoshida S, Ojima F, Ishiguro Y, et al. Hericenones C, D and E, stimulators of nerve growth factor (NGF)–synthesis, from the mushroom *Hericium erinaceum*. *Tetrahedron Lett* 1991;32(35):4561–4.
- [4] Kawagishi H, Ando M, Shinba K, Sakamoto H, Yoshida S, Ishiguro Y, et al. Chromans, hericenones F, G, and H, from the mushroom *Hericium erinaceum*. *Pytochemistry* 1993;32:175–8.
- [5] Kawagishi H, Shimada A, Shirai R, Okamoto K, Ojima F, Sakamoto H, et al. Erinacine A, B and C, strong stimulators of nerve growth factor (NGF)–synthesis from the mycelia of *Hericium erinaceum*. *Tetrahedron Lett* 1994;35(10):1569–72.
- [6] Kawagishi H, Shimada A, Shizuki K, Mori H, Okamoto K, Sakamoto H, et al. Erinacine D, a stimulator of NGF-synthesis, from the mycelia of *Hericium erinaceum*. *Heterocycl Commun* 1996;2:51–4.
- [7] Kawagishi H, Shimada A, Hosokawa S, Mori H, Sakamoto H, Ishiguro Y, et al. Erinacines E, F and G, stimulators of nerve growth factor (NGF)–synthesis, from the mycelia of *Hericium erinaceum*. *Tetrahedron Lett* 1996;37:7399–402.
- [8] Lee EW, Shizuki K, Hosokawa S, Suzuki M, Suganuma H, Inakuma T, et al. Two novel diterpenoids, erinacines H and I from the mycelia of *Hericium erinaceum*. *Biosci Biotechnol Biochem* 2000;64:2402–5.
- [9] Furukawa Y, Furukawa S, Satoyoshi E, Hayashi K. Catecholamines induce an increase in nerve growth factor content in the medium of mouse L-M cells. *J Biol Chem* 1986;261(13):6039–47.
- [10] David LS, Louis FR. Studies on the expression of the β nerve growth factor (NGF) gene in the central nervous system: level and regional distribution of NGF mRNA suggest that NGF functions as a trophic factor for several distinct populations of neurons. *Proc Natl Acad Sci USA* 1986;83:2714–8.
- [11] Lerea LS, McCarthy KD. Astroglial cells in vitro are heterogeneous with respect to expression of the α_1 -adrenergic receptor. *Glia* 1989;2:135–47.
- [12] Aoki C. β -Adrenergic receptor receptors: astrocytic localization in the adult visual cortex and their relation to catecholamine axon terminals as revealed by electron microscopic immunocytochemistry. *J Neurosci* 1992;12:781–92.
- [13] Furukawa S, Furukawa Y, Satoyoshi E, Hayashi K. Regulation of nerve growth factor synthesis/secretion by catecholamine in cultured mouse astroglial cells. *Biochem Biophys Res Commun* 1987;147:1048–54.

- [14] Zafra F, Lindholm D, Castren E, Hartikka T, Thoenen H. Regulation of brain-derived neurotrophic factor and nerve growth factor mRNA in primary cultures of hippocampal neurons and astrocytes. *J Neurosci* 1992;12:4793-9.
- [15] Schwartz JP, Nishiyama W, Wilson D, Taniwaki T. Receptor-mediated regulation of neuropeptide gene expression in astrocytes. *Glia* 1994;11:185-90.
- [16] Rainbow TC, Passone B, Wolf BB. Quantitative autoradiography of β_1 and β_2 adrenergic receptors in rat brain. *Proc Natl Acad Sci USA* 1984;181:1585-9.
- [17] Morgane PJ, Austin-LaFrance R, Bronzino J, Tonkiss J, Diaz-Cintra S, Cintra L, et al. Prenatal malnutrition and development of the brain. *Neurosci Biobehav Rev* 1993;17:91-128.
- [18] Dreyfus CF. Neurotransmitter and neurotrophins collaborate to influence brain development. *Perspect Dev Neurobiol* 1998;5:389-99.
- [19] Friedman WJ, Ibanez CF, Hallbook F, Persson H, Cain LD, Dreyfus CF, et al. Differential actions of neurotrophins in the locus coeruleus and basal forebrain. *Exp Neurol* 1993;119:72-8.
- [20] Swanson LW. The locus coeruleus: a cytoarchitectonic; Golgi and immunohistochemical study in the albino rat. *Brain Res* 1976;110:39-56.
- [21] McKinney M, Coyle JT, Hedreen TC. Topographic analysis of the innervation of the rat neocortex and hippocampus by the basal forebrain cholinergic system. *J Comp Neurol* 1983;217:103-21.
- [22] Coyle JT, Price DL, de Long MR. Alzheimer's disease: a disease of cortical cholinergic innervation. *Science* 1983;219:1184-9.
- [23] Chan-Palay V. Alteration in the locus coeruleus in dementias of Alzheimer's and Parkinson's disease. *Prog Brain Res* 1991;88:625-30.
- [24] German DC, Manaye KF, White III CL, Woodward DJ, McIntire DD, Smith WK, et al. Disease-specific patterns of locus coeruleus cell loss. *Ann Neurol* 1992;32:667-76.