Repeated Injections of Nicergoline Increase the Nerve Growth Factor Level in the Aged Rat Brain

Takeshi Nishio^{1,†}, Nobuhiko Sunohara¹, Shoei Furukawa², Ichiro Akiguchi³ and Yukitsuka Kudo⁴

¹Department of Neurology, Musashi Hospital, National Center of Neurology and Psychiatry, 4-1-1, Ogawahigashi, Kodaira, Tokyo 187, Japan

²Department of Molecular Biology, Gifu Pharmaceutical University, 5-6-1, Mitahorahigashi, Gifu 502, Japan

³Department of Neurology, Faculty of Medicine, Kyoto University, Shogoin-Kawaharacho, Sakyo-ku, Kyoto 606, Japan

⁴Department of CNS Pharmacology, Tanabe Seiyaku Co., Ltd., Kawagishi 2-2-50, Toda 335, Japan

Received December 1, 1997 Accepted January 29, 1998

ABSTRACT—We studied whether nicergoline, clinically active in chronic cerebrovascular insufficiency, influences nerve growth factor (NGF) levels in the rat brain. In young Fischer rats, repeated intraperitoneal injections of nicergoline (0.3 and 1.0 mg/kg body weight) did not show any effects on frontal NGF contents determined by a highly sensitive enzyme immunoassay. In aged rats, 22-month-old, however, repeated injections of nicergoline (1.0 mg/kg body weight) induced a significant increase in the NGF level in the frontal region.

Keywords: Nerve growth factor, Nicergoline, Aged rat brain

Nerve growth factor (NGF), the best characterized neurotrophic factor, has been shown to promote growth, survival and differentiation of neurons (1), and its potential therapeutic use was obvious early in its investigation. It seems clinically significant to find drugs that are readily available for clinical use and can potentially increase NGF levels in the brain. Nicergoline, an ergot alkaloid derivative, which has α_1 -adrenoreceptor-blocking and calcium antagonistic properties, increases cerebral blood flow and improves hemodynamics and glucose metabolism in aged rats with cerebral ischemia (2–5), inhibits acetylcholinesterase activity in the rat brain (6), and corrects reduced choline acetyltransferase (ChAT) and muscarinic cholinergic receptor (MCR) activities in aged rat brain (7). Although the mechanisms remain unknown, the effects of nicergoline on ChAT and MCR activities were observed only in aged rats and not in young adult rats (7). NGF has trophic effects on the central cholinergic neurons in the basal forebrain (1), and amelioration of cholinergic activities with nicergoline observed in aged rats may involve activation of trophic support of NGF. Therefore, we studied whether nicergoline influences the NGF levels in young adult or aged rat brains using a

highly sensitive two-site enzyme immunoassay (EIA).

Two groups of adult Fischer/F344 rats (young group (n=24): 7-week-old male rats weighing 136 to 153 g and aged group (n=24): 22-month-old male rats weighing 412 to 505 g) were used. Each animal group was subdivided into three subgroups; the low-dose group (n=6) received an injection of the agent (0.3 mg/kg body weight) intraperitoneally once a day for 14 days, the high-dose group (n=6) received an injection of the agent (1.0 mg/kg body weight) intraperitoneally once a day for 14 days and the control group (n=6) received a saline injection (2 ml/kg body weight) intraperitoneally once a day for 14 days. Each animal was sacrificed 4 to 5 hr after the last injection.

Rats were killed with deep narcosis induced by diethyl ether, and the brains were preserved at -80°C in a deep freezer until sample preparation. The frozen brains were coronally sectioned at a plane including the bregma with a stainless razor blade at -10°C in a cold room. The frontal region of the cerebrum was defined as the region anterior to the plane except for the olfactory bulb. Included were the frontal cortex, cingulate cortex, pyriform cortex, caudate putamen, diagonal band of Broca and corpus callosum in the frontal region. NGF was extracted from the frontal region of each brain, and the NGF content was determined by a highly sensitive two-site EIA (8).

In control rats (injected saline for 14 days), the NGF

[†] Present address for correspondence: Department of Integrative Brain Science, Faculty of Medicine, Kyoto University, Kyoto 606–8501, Japan

Fig. 1. The NGF levels in the defined frontal region of the young (7-week-old, ▨) and aged (22-month-old, ▩) Fischer rats injected with saline (control), low dose of nicergoline (0.3 mg/kg body weight, N-0.3) or high dose of nicergoline (1.0 mg/kg body weight, N-1.0). The values are shown as the mean $\pm$ S.E. Note the frontal NGF level of the aged rats injected with 1.0 mg/kg body weight of nicergoline was higher than that of the aged control rats. Statistical analyses were performed with pairwise multiple comparisons of Tukey-Kramer: * $P < 0.05$.

level in the frontal region was slightly decreased in the aged rats (144.00 ± 22.93 pg/g-tissue weight, mean $\pm$ S.E.), but was not significantly different from that in the young rats (161.43 ± 43.17) (Fig. 1). The frontal NGF levels were decreased with increasing doses of nicergoline in the young rats, although there was no significant difference. However, the frontal NGF levels were increased with increasing doses of nicergoline in aged rats (Fig. 1). The aged rats injected with nicergoline at 1.0 mg/kg body weight showed a significantly higher frontal NGF level than the aged control rats. Statistical analyses were done by the pairwise multiple comparisons of Tukey-Kramer; differences were considered significant when $P < 0.05$ (Fig. 1).

In this study, repeated intraperitoneal injections of nicergoline at 1.0 mg/kg body weight increased the NGF level in the defined frontal region in aged rats, but not in young rats.

The doses of nicergoline used in this study, 0.3 and 1.0 mg/kg body weight, are similar to the doses used in the other studies (3, 5, 7) and thus seem to be appropriate.

Nicergoline has pharmacological effects such as blocking α_1 -adrenergic receptor and calcium channels (2, 4, 5), improving hemodynamics and glucose metabolism (3), inhibiting acetylcholinesterase activity (6), and correcting

reduced ChAT and MCR activities in aged rat brain (7).

The mechanisms involved in elevating the NGF level in the aged rat frontal region subsequent to nicergoline injections remain to be clarified.

Messenger RNA for α -adrenergic receptor is expressed in the rat cerebral cortex (9), and activation of α -adrenoreceptor in vascular smooth muscle cells elevated NGF secretion (10). A calcium channel blocking agent eliminated the kainate-induced increase of NGF mRNA in the hippocampal neurons (11). These findings argue against the hypothesis that elevation of the NGF level involves α_1 -adrenergic receptor blocking or calcium channel blocking with nicergoline. In this study, the frontal NGF levels were slightly but not significantly decreased with increasing doses of nicergoline in the young rats. Therefore, the enhanced NGF level in the rat frontal region with nicergoline appears unlikely to be induced through α_1 -adrenergic receptor blocking or calcium channel blocking.

Nicergoline increased the frontal NGF level only in aged rats. With aging, the number of neurons decreases and the relative number of the glial cells increases, and reactive astroglial proliferations are noted with neuronal losses in the aged brain. Nicergoline enhances catecholamine turnover in the brain (12) and catecholamines

increase NGF mRNA in cultured astroglial cells (13, 14). Cultured astroglial cells are not in a resting state, but may relate to reactive states *in vivo*. Therefore, NGF production might be upregulated in reactive astroglial cells with nicergoline through enhanced catecholamine turnover. Though the detailed mechanisms of elevating the frontal NGF level in aged rats with nicergoline are unknown, the evidence that nicergoline ameliorated the activities of cholinergic neurons only in aged rats and not in young adult rats (7) could be explained by the present finding that nicergoline elevated the NGF level only in the aged rats, because NGF is known to potentiate cholinergic neuronal activities.

From the neurotrophic or neuroprotective perspective, the increase in NGF protein in the brain may be therapeutically beneficial for elderly individuals whose brain neurons are progressively dying with aging. It seems important to find agents that can potentially increase the protein levels of neurotrophins in the brain.

REFERENCES

- 1 Thoenen H: The changing scene of neurotrophic factors. *Trends Neurosci* **14**, 165–170 (1991)
- 2 Arcari G, Dorigotti L, Fregnan GB and Glasser AH: Vasodilating and alpha-receptor blocking activity of a new ergoline derivative. *Br J Pharmacol* **34**, 700 (1968)
- 3 Le Poncin-Lafitte M, Grosdemouge C, Duterte D and Rapin JR: Simultaneous study of hemodynamic, metabolic and behavioral sequelae in a model of cerebral ischemia in aged rats; effects of nicergoline. *Gerontology* **30**, 109–119 (1984)
- 4 Heitz C, Descombes JJ, Miller RC and Stoclet JC: α -Adrenoreceptor antagonistic and calcium antagonistic effects of nicergoline in the rat isolated aorta. *Eur J Pharmacol* **123**, 279–285 (1986)
- 5 Takahashi K and Akaike N: Nicergoline inhibits T-type Ca^{2+} channels in rat isolated hippocampal CA1 pyramidal neurons. *Br J Pharmacol* **100**, 705–710 (1990)
- 6 Matsuoka Y, Fukushima T, Iwata H, Matsumoto M, Shibutani Y, Kawashima K, Kudo Y and Ishida R: Inhibitory action of nicergoline and its major metabolites on acetylcholinesterase activity in rat and mouse brain. *In Basic, Clinical, and Therapeutic Aspects of Alzheimer's and Parkinson's Disease*, Edited by Nagatsu T, Fisher A and Yoshida H, Vol 2, pp 415–419, Plenum Press, New York (1990)
- 7 Ogawa N, Asanuma M, Hirata H, Kondo Y, Kawada Y and Mori A: Cholinergic deficits in aged rat brain are corrected with nicergoline. *Arch Gerontol Geriatr* **16**, 103–110 (1993)
- 8 Nishio T, Akiguchi I and Furukawa S: Detailed distribution of nerve growth factor determined by a highly sensitive enzyme immunoassay. *Exp Neurol* **116**, 76–84 (1992)
- 9 Lomasney JW, Cotecchia S, Lorenz W, Leung W-Y, Schwinn DA, Yang-Feng TL, Brawnstein M, Lefkowitz RJ and Caron MG: Molecular cloning and expression of the cDNA for the α_{1A} -adrenergic receptor. *J Biol Chem* **266**, 6365–6369 (1991)
- 10 Tuttle JB, Etheridge R and Creedon DJ: Receptor-mediated stimulation and inhibition of nerve growth factor secretion by vascular smooth muscle. *Exp Cell Res* **208**, 350–361 (1993)
- 11 Zafra F, Lindholm D, Castren E, Hartikka J and Thoenen H: Regulation of brain-derived neurotrophic factor and nerve growth factor mRNA in primary cultures of hippocampal neurons and astrocytes. *J Neurosci* **12**, 4793–4799 (1992)
- 12 Moretti A, Carfagna N, Caccia C, Carpentieri M, Amico A, Marchi G and Trunzo F: Neurochemical effects of ergoline derivatives. *In Proof of Therapeutical Effectiveness of Nootropic and Vasoactive Drugs; Advances in Clinical and Experimental Nicergoline Research*, Edited by Heidrich H, pp 103–110, Springer-Verlag, Berlin, Heidelberg, New York and Tokyo (1985)
- 13 Furukawa S, Furukawa Y, Satoyoshi E and Hayashi K: Regulation of nerve growth factor synthesis/secretion by catecholamine in cultured mouse astroglial cells. *Biochem Biophys Res Commun* **147**, 1048–1054 (1987)
- 14 Furukawa Y, Furukawa S, Satoyoshi E and Hayashi K: Catecholamines increase nerve growth factor mRNA contents in both mouse astroglial cells and fibroblasts cells. *FEBS Lett* **247**, 463–467 (1989)