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NGF LEVEL IS NOT DECREASED IN THE SERUM, BRAIN-SPINAL FLUID, HIPPOCAMPUS, OR PARIETAL CORTEX OF INDIVIDUALS WITH ALZHEIMER'S DISEASE

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SUMMARY: Although the cause of Alzheimer's disease (AD) is unknown, nerve growth factor (NGF) has gained attention as a therapeutic agent for the disease. Because NGF maintains the magnocellular cholinergic neurons that are damaged in AD, research interests have been focused on the change in NGF level in patients with AD. This is the first reported study in which human NGF levels were accurately measured and compared between normal and AD samples. We measured NGF levels using enzyme immunoassay (EIA) system for human NGF and found no difference in NGF level in serum, brain-spinal fluid, or brain (hippocampus and parietal cortex) obtained from normal people and patients with AD. These results suggest that a decrease in the NGF level is not a causative factor of AD.

Nerve growth factor (NGF) is well known as a neurotrophic factor that stimulates the differentiation and maintains the survival of sympathetic and certain sensory neurons in the peripheral nervous system (PNS) (1-4), and of magnocellular cholinergic neurons of the basal forebrain in the central nervous system (CNS) (2,5). In Alzheimer's disease (AD) memory loss and neuronal cell death of basal forebrain cholinergic neurons were reported (6-9). These findings suggest that if the function of these cells could be maintained the deterioration of cognitive function that occurs in AD might be showed down or lessened. Although NGF has been proposed as a potential therapeutic agent for AD, there is no evidence to indicate that this disease is associated with a significant deficiency in NGF. So far there is little information on

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Abbreviations: AD, Alzheimer's disease; NGF, nerve growth factor; EIA, enzyme immunoassay; PNS, peripheral nervous system; CNS, central nervous system; BDNF, brain-derived neurotrophic factor; NT-3, neurotrophin-3.

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the presence of NGF in human brain and other human samples, although it has been reported that NGF mRNA (10) and NGF receptor mRNA levels (11) are unchanged in AD patients. Thus, to compare the NGF level in AD and control individuals, we examined samples of human serum, brain-spinal fluid, and brain (hippocampus and parietal cortex) by an EIA for human NGF.

MATERIALS AND METHODS

Materials

Recombinant human NGF (12) and anti-human NGF antiserum (13) were prepared as previously reported. All other chemicals were reagent grade.

Two-site enzyme immunoassay (EIA) for human NGF

The EIA was based on the sandwiching of antigen between anti-human NGF antibody IgG coated on polystyrene plates and biotinylated anti-human NGF antibody IgG. The bound antibody complex was quantified with streptavidin-linked β-D-galactosidase (13).

Preparation of serum samples

Blood was collected and was allowed to clot by standing for 2 hr at room temperature and was then kept overnight at 4°C. Serum samples were obtained after centrifugation of the coagulated blood. We incubated 10% serum in 1M guanidine hydrochloride for 5 hr at 37°C, and then subjected it to EIA system (14).

Preparation of spinal fluid samples

Brain-spinal fluid was collected and stored at -80°C until used. Samples were diluted with cold 0.1M Tris-HCl buffer, pH 7.6, containing 1M NaCl, 2% BSA, 2mM ethylenediamine tetraacetic acid (disodium salt), 80 trypsin inhibitory units of aprotinin/liter, and 0.02% NaN_3 (Buffer A) at 20% volume per volume.

Preparation of homogenate from human brain

The tissue was frozen on dry ice and stored at -80°C. The samples were sonicated in cold buffer A at 5% wet tissue weight per volume. The solution was centrifuged at 40,000xg for 30 min, and the supernatant obtained was assessed for NGF content.

RESULTS AND DISCUSSION

NGF has been well investigated in the CNS and PNS of rodents and non-human primates. On the contrary, only a few studies on human NGF have been performed, basically for two reasons: it is difficult to obtain human NGF in a sufficient amount to raise antibody; and the EIA for mouse NGF, which was the only method for the assay of human NGF, shows low immunocrossreactivity between mouse NGF and human NGF due to the differences in amino acid sequences. Recently, however, recombinant human NGF was prepared (12); and so we raised anti-human NGF antibody against it and established a sensitive EIA system for this NGF (13). Our assay system can detect human NGF quantitatively at a concentration as low as 0.3 pg/ml and dose not detect other neurotrophic factors like brain-derived neurotrophic factor

(BDNF) or neurotrophin-3 (NT-3) when these factors are present below a concentration of 100 ng/ml. Thus, our antibody against human NGF has high specificity.

To investigate the possibility of diagnosis of dementia in its early stage, we measured the NGF level in serum. We reported earlier that NGF is present in rat serum and that it forms a complex with α_2-macroglobulin, the formation of which inhibits the immunoreactivity between NGF and its antibody (14). We succeeded in measurement of rat serum NGF by EIA after the treatment of the serum with 1M guanidine hydrochloride. Because human NGF also forms a complex with α_2-macroglobulin, we treated human serum with 1M guanidine hydrochloride before application to our EIA. The level of human NGF in serum samples from normal individuals in their fifties was 5.57 $\pm$ 0.57 pg/ml; for AD patients, 6.12 $\pm$ 0.52 pg/ml (Fig.1). The recovery of 100 pg/ml of human NGF added to samples was about 90%, and so all the values were revised. The level of NGF in serum was not significantly different in AD compared with that of age-matched control samples. However, the level of NGF in serum of normal individuals in their twenties and normals of unknown age were 12.05 $\pm$ 1.30 pg/ml and 11.83 $\pm$ 0.63 pg/ml, respectively. This finding indicates that the level of NGF in serum decreases with increasing age. Serum NGF may be derived from blood vessels, as blood vessels are tissues rich in sympathetic neurons, which neurons are known to be one of the dominant sites of NGF

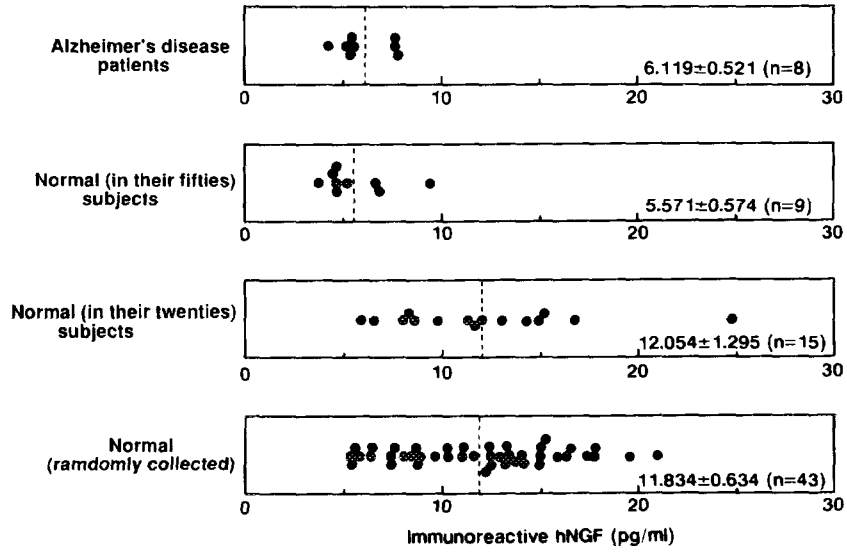

Fig.1. Levels of immunoreactive human NGF in serum.
Each point indicates the mean of triplicate assays. Each vertical dotted line indicates the mean value. Each value at the right indicates the mean $\pm$ SE.

synthesis. We reported that the NGF level in rat serum might reflect the demand for this factor during establishment of the peripheral nervous system (14). Saide et al. detected immunoreactive NGF in normal human serum (60-80 ng/ml) and reported 3-6-fold higher levels in patients with Paget's disease than in controls using an EIA for mouse NGF (15). Heinrich and Meyer detected NGF in three human sera (800 ± 60 pg/ml, 1,400 ± 110 pg/ml, and 130 ± 15 pg/ml, respectively) using anti-mouse NGF antibody (16). These serum levels are higher than those of our results.

Next we investigated the NGF level in brain-spinal fluid samples from normal controls and patients with neuronal disease (Fig.2). The level in normal brain-spinal fluid was 7.27 ± 3.96 pg/ml; and that in patients with neuronal disease was almost the same (7.59 ± 2.57 pg/ml).

AD is characterized by structural changes (neuronal cell death and the presence of amyloid plaques and neurofibrillary tangles) in several cortical and subcortical areas, especially in the association centers, hippocampus, and the basal forebrain, the latter of which is rich in cholinergic magnocellular neurons. A strong correlation also exists between the reduction in cholinergic marker activity and the degree of dementia (17,18). Although the level of NGF mRNA is not significantly changed in hippocampal neurons in AD compared with that in control samples (10), we also investigated the NGF levels in hippocampus and parietal cortex, in which NGF is synthesized in vivo, from normal controls or AD patients (Fig.3). There was no difference in NGF level between them. The recovery of brain-spinal fluid NGF and brain NGF was almost 100%. Phillips et al. reported that the *in situ* hybridization signal for BDNF, but not

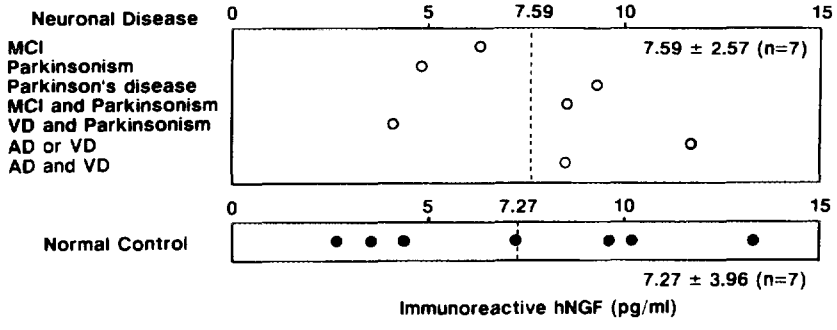

Fig.2. Levels of immunoreactive human NGF in brain-spinal fluid.
Each point indicates the mean of triplicate assays. Each vertical dotted line indicates the mean value. Abbreviations : MCI, Multiple Cerebral Infraction; VD, Vascular Dementia; AD, Alzheimer's disease.

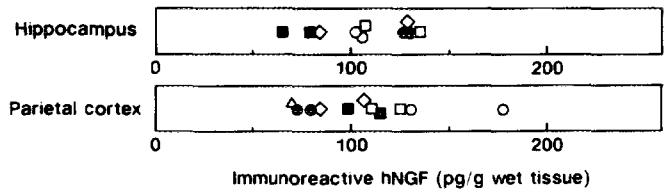

Fig.3. Levels of immunoreactive human NGF in hippocampus and parietal cortex. Each point indicates the mean of triplicate assays. Open and closed symbols indicate the values for Alzheimer's disease and normal control, respectively. Same individuals are indicated by the same symbols, e.g., o and o for hippocampus mean two samples from different areas of hippocampus in patient "o".

that for NGF nor that for NT-3, was decreased in samples of hippocampus from donors with AD (19). These findings, as well as our current ones, suggest that there is little relationship between NGF level and AD. In spite of this, NGF was anticipated as one of the therapeutic agents for the disease because pharmacological study on NGF has shown that continuous intracerebral infusion of NGF could partly reverse the cholinergic cell body atrophy and improve retention of a spatial memory task in behaviorally impaired aged rats (20). Olson administrated mouse NGF to a patient with AD by the intracerebroventricular route and observed some positive recuperations (21). Recently NGF conjugated to an antibody to the transferrin receptor passed through the blood-brain barrier after peripheral injection, and this conjugated NGF increased the survival of cholinergic neurons (22). Even though the NGF level is not decreased in AD patients, elevation of the level by exogenous NGF may possibly maintain the cholinergic neurons. Additional studies are needed to determine the detailed relationship between BDNF and AD and for the application of neurotrophic factor to neuronal disease.

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