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Memory impairment and morphological changes in rats induced by active fragment of anti-nerve growth factor-antibody

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Treatment of rats with a specific Fab' fragment of anti-nerve growth factor (NGF)-antibody (anti-NGF, 12, 120 and 400 μg/4 weeks, i.c.v.) impaired their learning ability. The distance of swimming of anti-NGF-treated rats in a water maze was shortened more slowly by training than that of control rats. Anti-NGF treatment altered the staining of nuclei of cells in the hippocampus, parietal cortex and dentate gyrus with hematoxylin. It is suggested that the anti-NGF-induced amnesia could be due to change in nuclear morphology.

Nerve growth factor (NGF) is important in the development and maintenance of peripheral sympathetic neurons (1). Injections of anti-NGF-antiserum into neonatal mammals has been shown to result in irreversible destruction of sympathetic ganglia (2). Moreover, recent anatomical, behavioral, and biochemical studies have suggested a possible role for NGF in the central nervous system (3-8). However, there is no direct evidence that altered levels of NGF cause either normal aging or neurodegenerative processes such as Alzheimer's disease. Furthermore, intraventricular and intracortical injections of anti-NGF-antiserum did not have adverse effects on basal forebrain cholinergic neurons (9). It is also unknown whether deficiency of NGF impairs learning and memory. We

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report here that continuous intracerebral infusion of a specific Fab' fragment of anti-NGF-antibody (anti-NGF) into adult rats for four weeks impaired their abilities to learn and remember a water maze.

METHODS

The Fab' fragment of anti-NGF-antibody was infused into the cerebral ventricle of rats to increase its penetration and wide distribution in the brain and to remove NGF completely. For this we used male Wistar rats weighing about 250 g of 50-56 days old at the start of the study. Cannulae attached to modified mini-osmotic pumps for continuous infusion of solution for two weeks were implanted into the lateral ventricles (Bregma: A;-0.5 mm, L;1.5 mm, H;4.5 mm) of all the rats under anaesthesia with sodium pentobarbital according to the coordinates in the atlas of Paxinos and Watson (10). The rats were then divided into 4 groups, a control and three anti-NGF treated groups. For the anti-NGF-treated rats, the pump was filled with artificial cerebrospinal fluid (CSF) containing 30, 300 or 1000 μg/ml of anti-NGF and 1 mg/ml of bovine serum albumin (BSA). The estimated anti-NGF doses were 6, 60 and 200 μg/2 weeks. For the control rats, the pump was filled with CSF containing the Fab' fragment of normal IgG and BSA. The pumps were replaced by new ones containing the same solutions after 15 days.

The Fab' fragment of anti-NGF was prepared from a rabbit IgG fraction of anti-NGF-antiserum (11). The biological activity of anti-NGF was judged by its ability to neutralize NGF-induced fiber outgrowth of PC12 cells. The Fab' fragment of normal IgG was prepared similarly to that of the Fab' fragment of anti-NGF-antibody.

A water maze was used to investigate the amnesic effect of anti-NGF. The rats were put in the maze once a day (one trial a day) during the anti-NGF infusion period at 10:00-16:00 between day 8 and day 14 (2nd week), and between day 22 and day 28 (4th week) after the start of infusion. The water maze was illuminated by two lamps (50 W).

The water maze (12) consisted of a circular water tank (140 cm in diameter) with a transparent platform (10 cm in diameter) in a constant position in the middle of one quadrant inside the tank, equidistant from the center and edge of the pool. The tank was filled with water of approximately 23°C to 2 cm above the top of the platform. The tank was kept in a large test room, with many cues around it, which were visible from within the pool and could be used by the rat for spatial orientation. The positions of the cues were kept constant throughout the training period. In the 4th week the cues were rearranged, keeping the position of the platform fixed. In each training session, the rat was placed in the water facing the wall of the pool at each of five starting positions, but the sequence of the starting positions was selected at random. If the rat was unable to find the platform within 300 sec, the training session was terminated. The trace of movement and the distance of swimming in the water-maze were monitored with a TV camera (BTA-2A, Muromachi Co.) and analyzed by computer.

For examination of changes in nuclear morphology induced by anti-NGF, the rats were perfused transcardially with a solution of formaldehyde (3.5 %) in phosphate buffer on day 31 after the start of anti-NGF infusion. The brain was then removed and embedded in paraffin. Sagittal sections (4 μm in thickness) of a 2.4 mm wide midline portion of the brain were cut from the blocks on a microtome, stained with hematoxylin solution (Merck) and examined by light microscopy (BHT-322, Olympus, Japan).

Data for rats in which the cannula was not connected with the tube at the time of sacrifice were omitted in evaluation of results.

RESULTS

Fig.1 shows typical traces of the movement of a control rat infused with the Fab' fragment of normal IgG and rats treated with anti-NGF (200 μg/2 weeks and 400 μg/4 weeks, i.c.v.). The distance of swimming of control rats decreased more rapidly day by day than those of the anti-NGF-treated rats. By the end of training, the distance of swimming of control rats had decreased to approximately 173 cm per training, but that of anti-NGF (400 μg/4 weeks)-treated rats was approximately 389 cm per training.

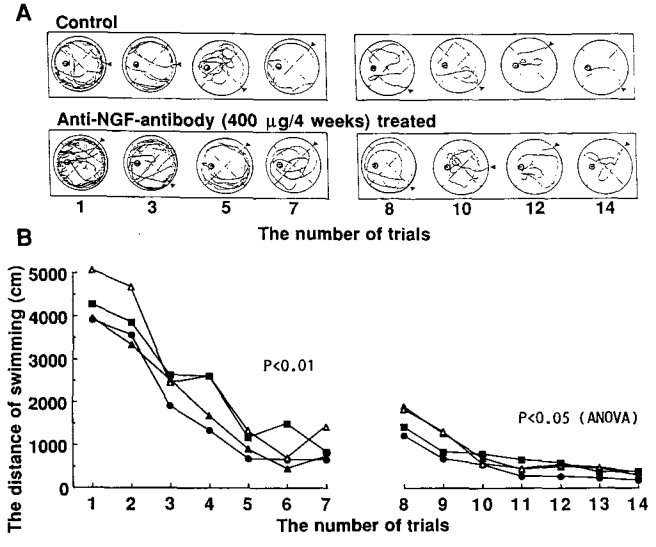
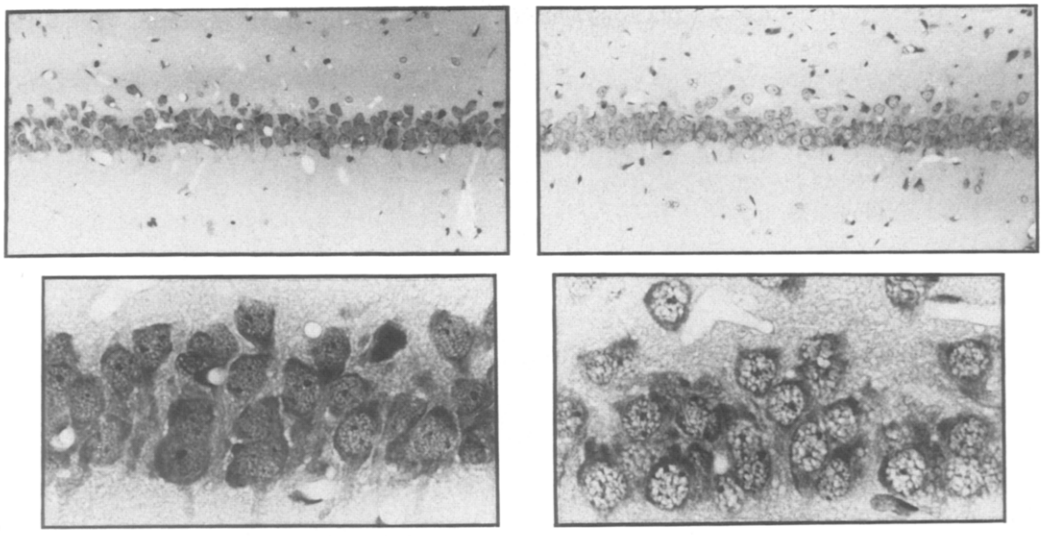


Fig.1. Effect of chronic infusion of an active Fab' fragment of anti-NGF-antibody (anti-NGF) on the learning and memory of rats of a water maze task. Traces of movement (Fig. A) and distances of swimming (Fig. B) of rats were recorded in the 2nd and 4th weeks after the start of infusion. The traces of movement in the water maze are from the starting point to the platform. Each group consisted of 7-10 rats, the groups being: ○ ;the control group; and groups treated with anti-NGF at, ●, 12 μg/4 weeks; ▲, 120 μg/4 weeks, and ■, 400 μg/4 weeks.



Control

Anti-NGF-antibody treated
(400 μg/4 weeks)

Fig.2. Changes in nuclei of cells in the hippocampal CA1 subfield induced by chronic infusion of an active Fab' fragment of anti-NGF-antibody.

Statistical analysis of data for the 2nd and 4th weeks by two-way ANOVA showed differences between the groups in both evaluations throughout the test weeks (2nd week, $F(3, 224)=5.19$, $p<0.01$: 4th week, $F(3, 252)=2.68$, $p<0.05$).

In the hippocampal CA1 subfield (Fig. 2), inner layer of the parietal cortex (data not shown) and dentate gyrus (data not shown) of the anti-NGF-treated rats, the nuclei of cells were unevenly stained with hematoxylin. This uneven staining was not observed in other regions of the brain of anti-NGF-treated rats, or in rats treated with the Fab' fragment of normal IgG.

DISCUSSION

The present study showed that chronic anti-NGF-infusion caused dose-dependent impairment of the memory of rats for a water maze. Anti-NGF antibody may alter nuclear morphology because we found that it altered the staining of nuclei in the hippocampus, parietal cortex and dentate gyrus with hematoxylin. It may also impair other functions of NGF such as induction of hypertrophy, membrane effects, increases in second messengers, stimulation of transports, and changes in protein phosphorylations.

Recent studies have demonstrated roles of target-derived trophic factors in maintenance of the basal forebrain cholinergic projection system

(13-15), and have suggested that NGF may be of particular importance in this trophic regulation (1). Since decreased levels of NGF and its messenger RNA have been found in aged rat brain (16), the present results suggest that the age-dependent structural and functional deterioration of forebrain cholinergic neurons, implicated in Alzheimer's disease, may be due to either the loss or dysfunction of NGF induced by auto-immunization. The rat model developed in the present work should be useful for further studies on physiological changes due to a deficiency of NGF in the adult central nervous system.

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REFERENCES

1) Yu, M.Y.W., Lakshmanan, J. and Guroff, G., In M.B.H. Youdim, W. Lovenberg, D.F. Sharman and J.R. Lagnado (Eds), Essay in Neurochemistry and Neuropharmacology, Wiley, New York, 3:33-48, 1978.
2) Johnson, E.M.Jr., Gorin, P.D., Brandeis, L.D. and Pearson, J., Science, 210:916-918, 1980.
3) Fischer, W., Wictorin, K., Björklund, A., Williams, L.R., Varon, S. and Gage, F.H., Nature, 329:65-68, 1987.
4) Furukawa, S., Furukawa, Y., Satoyoshi, E. and Hayashi, K., Biochem. Biophys. Res. Commun., 142:395-402, 1987.
5) Furukawa, S., Furukawa, Y., Satoyoshi, E. and Hayashi, K., Biochem. Biophys. Res. Commun., 147:1048-1054, 1987.
6) Johnson, E.M.Jr. and Taniuchi, M., Biochem. Pharmacol., 36:4189-4195, 1987.
7) Lewis, M.E., Lakshmanan, J., Nagaiah, K., MacDonnell, P.C. and Guroff, G., Proc. Natl. Acad. Sci. USA., 75:1021-1023, 1978.
8) Lewis, M.E., Brown, R.M., Brownstein, M.J., Hart, T. and Stein, D.G., Brain Res., 176:297-310, 1979.
9) Gnahn, H., Hefti, F., Heumann, R., Schwab, M.E. and Thoenen, H., Devel. Brain Res., 9:45-52, 1983.
10) Paxinos, G. and Watson, C., The rat brain in stereotaxic coordinates., Academic Press Australia, 1986.
11) Hayashi, K., Nomoto, H., Kurobe, M., Nishimuro, S., Hiratani, H. and Furukawa, S., Biochem. Inter., 10:839-845, 1985.
12) Morris, R., J. Neurosci. Meth., 11:47-60, 1984.
13) Hefti, F., J. Neurosci., 6:2155-2162, 1986.
14) Kromer, L.F., Science, 235:214-216, 1987.
15) Williams, L.R., Varon, S., Peterson, G.M., Wictorin, K., Fischer, W., Bjorklund, A. and Gage, F.H., Proc. Natl. Acad. Sci. USA., 89:9231-9235, 1986.
16) Lärkfors, L., Ebendal, T., Whittemore, S.R., Persson, H., Hoffer, B. and Olson, L., Mol.Brain Res., 3:55-60, 1987.