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Short communication

Nerve growth factor improves evoked potentials and long-term potentiation in the dentate gyrus of presenile rats

Jorge A. Bergado *, Andrés A. Gómez-Soria, Reynier Cruz, Caridad I. Fernández

Centro Internacional de Restauración Neurológica, Ave. 25 #15805, Playa, 12100 La Habana, Cuba

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Abstract

Chronic infusion of nerve growth factor (NGF, 1.2 μg/day) for 14 days to presenile rats (17 months at the beginning of treatment) that showed an initial cognitive impairment led to an improved long-term potentiation in the dentate gyrus. Both the relative increase of the slope of the population excitatory postsynaptic potential and that of the population spike were enhanced by NGF pretreatment after long-term potentiation induction at 400 Hz. The treatment was also able to increase the diminished baseline amplitude of the population spike, an effect not seen when the treatment was applied to older animals [Bergado, J., Fernández, C.I., Gómez-Soria, A., González, O., 1997a. Chronic intraventricular infusion with NGF improves LTP in old cognitively-impaired rats. Brain Res. 770, 1–9] stressing the importance of an early start of trophic therapy to achieve better results. © 1998 Elsevier Science B.V.

Keywords: NGF (nerve growth factor); Evoked potential; Long-term potentiation

1. Introduction

The ageing process is accompanied by a progressive reduction of memory capacity which has been attributed to a reduced cholinergic input to relevant brain areas, like the hippocampus (Bartus et al., 1982; Fischer et al., 1989). Nerve Growth Factor (NGF) exerts a trophic action on the basal forebrain cholinergic population (Hefti, 1986) and, in behavioral paradigms, improves cognition in aged rats (Fischer et al., 1987; Hellweg et al., 1990; Fernández et al., 1995).

Long-term potentiation has been proposed as a cellular model of synaptic plasticity and memory formation (Bliss and Lomo, 1973; Krug et al., 1990; Bliss and Collingridge, 1993), which is impaired by ageing (see review in Geinisman et al., 1995). We have previously shown that chronic intraventricular infusion with NGF ameliorates the impaired long-term potentiation shown by aged rats with cognitive impairments (Bergado et al., 1997a). This effect was, however, incomplete because the treatment showed no effect on the reduced baseline amplitude of the population spike, and long-term potentiation was brought to a level comparable to that of aged non-impaired rats, but not to that of young animals.

One possible way to improve the efficacy of NGF treatment is to start the infusion earlier. Based on this reasoning we have tried to assess in this paper whether the application of NGF to presenile rats showing a mild cognitive dysfunction would lead to better results than the application of the trophic substance to older animals.

2. Materials and methods

Male Sprague–Dawley rats, obtained as retired breeders from CENPALAB (Havana, Cuba), were tested every two months, starting at the age of 10 months, using a set of learning paradigms to discover early impairments of memory functions. At the age of sixteen months a subgroup of rats showed a light, but significant deterioration of memory in the Morris water maze (mean latency above two standard deviations that of young controls) and the animals were submitted to NGF treatment, starting at seventeen months. Three experimental groups were formed with these impaired presenile rats. A group (NGF, $n = 7$) received 14 days continuous infusion (1.2 μg/day) with murine 2.5S NGF purified at CIREN (Havana, Cuba) from mouse submaxillary glands. Another group (vehicle, $n = 4$) received saline solution. The substance delivery in both groups was carried out via Alzet 2002 miniosmotic pumps

* Corresponding author. Fax: +53-7-332420.

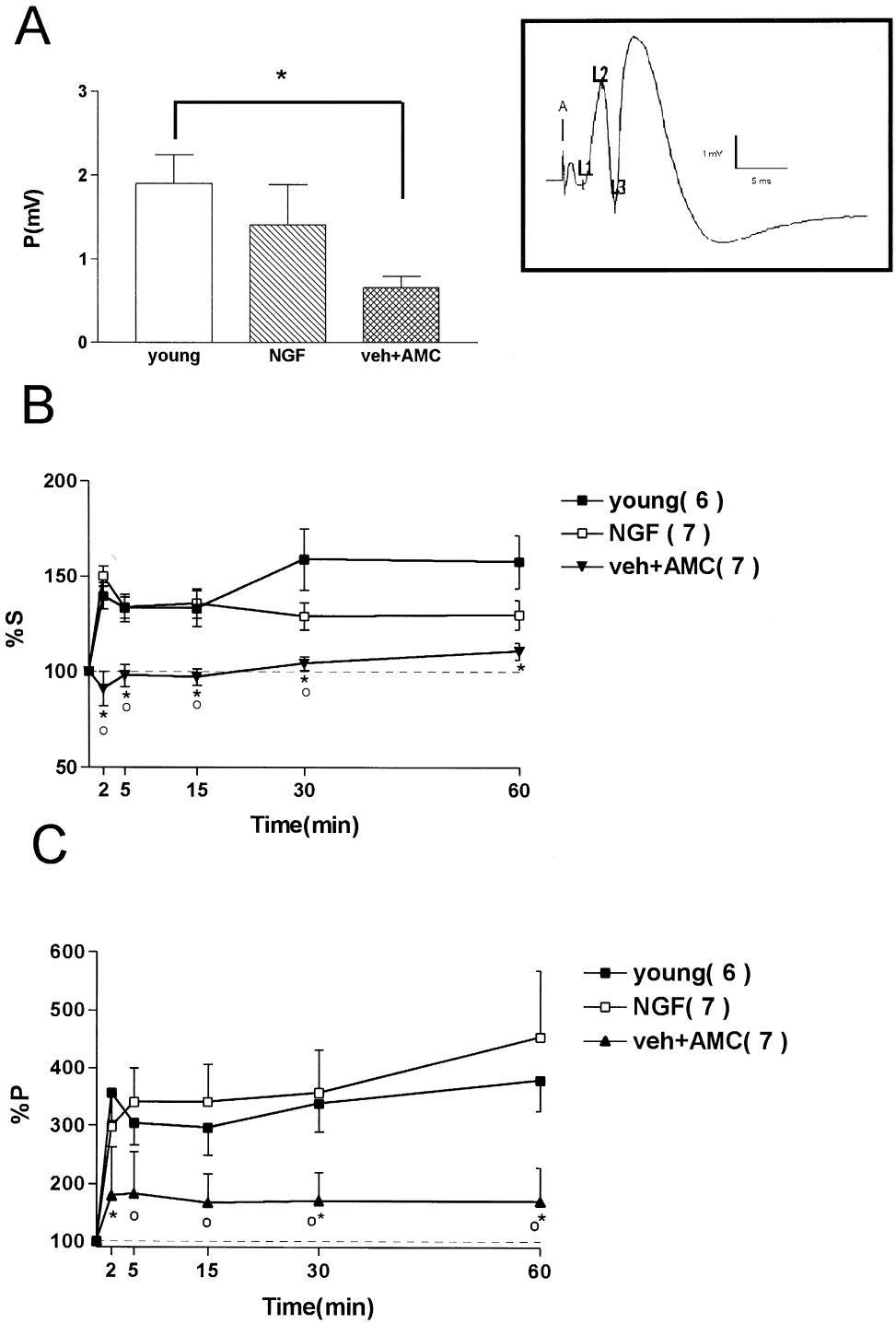

Fig. 1. (A) Baseline amplitude of the population spike in the experimental groups (mean ± S.E.M.). ∗ $P < 0.05$, Mann–Whitney's U test. (B) Time-course of the potentiation of the population EPSP (S). Values (mean ± S.E.M.) relative to the baseline pretetanization values (broken line at 100%) are shown. ∗Significant difference between the vehicle + age-matched control (veh + AMC) group with respect to the young group, °Significant difference between the veh + AMC group with respect to the NGF group. (C) Time-course of the potentiation of the population spike (P). Values (mean ± S.E.M.) relative to the baseline pretetanization values (broken line at 100%) are shown. ∗Significant difference between the veh + AMC group with respect to the young group; °Significant difference between the veh + AMC group with respect to the NGF group. In (B) and (C): $P < 0.05$, Mann–Whitney's U test. Insert: An evoked potential in the dentate gyrus obtained from a young animal. L1, L2 and L3 indicate the position of the cursors used for measuring the variables used in the present paper. The population spike amplitude was measured (mV) as the amplitude difference between L2 and L3. The EPSP slope was calculated (mV/ms) as the ratio between the amplitude difference between L1 and L2 and the time delay between the same points.

(Alzet, USA) connected to the right ventricle through an implanted cannula. Other impaired age-matched controls (age-matched controls, $n = 3$) received no treatment. Finally, a group of two months old animals (young, $n = 6$) was also evaluated as control. The vehicle-treated animals and the age-matched controls showed no differences in any of the measured variables, they where further considered as one single group (vehicle + age-matched controls, $n = 7$).

The electrophysiological evaluation was carried out under chloral hydrate (420 mg/kg) two weeks after the end of infusion. A monopolar recording electrode and a bipolar stimulating electrode were placed stereotaxically in the dentate gyrus and the perforant pathway respectively, at the coordinates (in mm) A: −3.8, L: 2.0, V: −3.5 for recording and A: −7.0, L: 4.0, V: −4.1 for stimulation. The final vertical position of the electrodes was adjusted under visual inspection of the evoked potentials to obtain the best sensitivity.

Each recorded potential consisted of the average of the responses to four consecutive stimuli at 0.2 Hz from which the amplitude of the population spike (P) was measured and the slope of the population excitatory postsynaptic potential (EPSP) was calculated as described in Fig. 1 insert. For stimulation monophasic, constant current square pulses (0.1 ms) were applied through a stimulus isolation unit. The stimulus intensities were individually determined and set at half maximal population spike for testing and quarter maximal population amplitude for the induction of long-term potentiation. The baseline values were obtained averaging three records with 1-min interval before tetanization. Tetanic stimulation to induce long-term potentiation consisted of ten trains of ten impulses each at 400 Hz with a 10-s intertrain interval. Test records after long-term potentiation induction were obtained at 2, 5, 15, 30 and 60 min after tetanization. The values of the population spike amplitude and of the EPSP in these records were expressed as percentages of the corresponding baseline values.

Narcosis was maintained with one third the initial dosage of chloral hydrate 2 h after the first bolus and every hour after that. The rectal temperature was monitored and kept between 36.6–37°C by using a radiant lamp.

3. Results

The first interesting result is that chronic NGF infusion to presenile rats seemed to increase the amplitude of the baseline population spike (Fig. 1A), an effect which was not seen when the infusion was started at later ages, e.g., 21 months (Bergado et al., 1997b). No differences in the baseline values of the EPSP were observed (young = 3.71 ± 1.23, NGF = 4.38 ± 1.73, vehicle + age-matched controls = 4.10 ± 0.30, all values in mV/ms, nonsignificant differences, mean ± S.E.M).

Like older cognitively impaired rats, presenile rats also showed an absent population EPSP potentiation (Fig. 1B). The treatment with NGF restored the initial potentiation of the EPSP although it seems to decline with time, suggesting that NGF might have enabled the induction of long-term potentiation of this variable, but not its long-term maintenance.

The present results show (Fig. 1C) that presenile rats with cognitive impairment are able to develop a potentiation of the population spike amplitude, as shown in the first records following tetanic stimulation, although the potentiation was diminished in amplitude with respect to that of young and NGF-treated animals.

4. Discussion

An important sign of ageing seems to be the inability of rats to maintain long-term potentiation and to express the kindling phenomenon (e.g., Barnes, 1994). The cognitive impairment shown by aged animals has been attributed to a reduction in the cholinergic innervation to relevant areas like the hippocampus (Bartus et al., 1982; Fischer et al., 1989). This impairment could be causally related to the absent EPSP potentiation observed among presenile rats showing an initial deterioration of their memory functions. A similar impairment of the EPSP was observed among young rats bearing lesions of the fimbria-fornix (Buzsaki and Gage, 1989; Bergado et al., 1997b), indicating that the improved induction of an EPSP potentiation after NGF might be related to the trophic action of this neutrophin on the cholinergic septal population (Fischer et al., 1987).

We have previously reported (Bergado et al., 1997a) a delayed potentiation of the population spike among aged rats with cognitive impairment. Attempting to explain the delayed potentiation shown by aged rats, we hypothesized that this delay was not likely to be dependent on cholinergic denervation because fimbria-fornix lesioned rats showed no impairment of population spike potentiation (Bergado et al., 1996, 1997b), and the possible role of a reduced glutamatergic transmission (McEntee and Crook, 1993) was considered instead. This appears to be an interesting time gradient of impairment of population spike potentiation that appears when one considers the absence of impairment in fimbria-fornix lesioned young rats, the present, but reduced potentiation of presenile rats and finally the delayed potentiation among aged rats. NGF treatment was able to normalize the altered population spike potentiation, both among aged (Bergado et al., 1997a) and presenile rats.

Considering the time elapsed between the end of the treatment and the electrophysiological evaluation, and the fact that NGF have no acute effects on neuronal plasticity (Ishiyama et al., 1991), the results reported in the present paper might be better explained in terms of trophic influences improving neuronal survival, stimulation of sprout-

ing and similar mechanisms leading to enhanced connectivity. If our assumption of a non-cholinergic nature of the population spike potentiation impairment among aged rats is correct, this might indicate that chronic NGF treatment can have effects other than increasing the survival of septal cholinergic neurons, which have been, until now, the only known action of this neurotrophin within the central nervous system (Hefti, 1986). In line with this reasoning is the report from Cellerino (1996) showing that measurable levels of TrkA-receptor mRNA are found in the hippocampal, opening thus the possibility of an autocrine action of NGF on the hippocampus itself.

Such an autocrine action might have also been involved in the increased amplitude of the baseline population spike amplitude in NGF-treated presenile rats. A reduced in the population spike amplitude has been attributed to a reduction in the cell density with ageing (Papatheodoropoulos and Kostopoulos, 1996). However, evidence of production of new granule cells have been reported in rats up to 18 months (Seki and Arai, 1995) raising the exciting perspective of an action of NGF, when applied early enough, to increase the number of neurons in the hippocampus. Of course, further research is required to assess this hypothesis.

Our results provide further evidence of the potential therapeutic usefulness of NGF in the treatment of age-related cognitive dysfunctions and stressed the importance of an early treatment to obtain better results.

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