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Rapid communication

BDNF concentrations are decreased in serum and parietal cortex in immunotoxin 192 IgG-Saporin rat model of cholinergic degeneration

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ABSTRACT

The neurotrophin brain-derived neurotrophic factor (BDNF) has been extensively studied because of its role in survival, differentiation and function of neurons undergoing degeneration in pathological conditions such as cholinergic neurons in Alzheimer's disease (AD). However, despite these evidences, the role of BDNF in these events is still matter of debate because central and peripheral BDNF levels are often found in opposite direction. Another puzzling factor is represented by pharmacological treatments known to cause alterations of BDNF peripheral levels. Thus, a pivotal issue would be to verify whether brain and serum BDNF changes are interconnected as well as the possibility that different stages of cholinergic degeneration are characterized by different changes in BDNF brain and serum levels.

With this in mind in this study we used a rat model of cholinergic degeneration based on intracerebroventricular (i.c.v.) injections of 192 IgG-Saporin and measured brain and serum BDNF concentrations by enzyme-linked immunosorbent assay (ELISA) at 3, 7 and 15 days from immunotoxin injection. We found that BDNF levels were reduced in parietal cortex and serum of Saporin-treated rats at 15 days from lesion. Moreover, a positive correlation between serum and parietal cortex was observed at 15 days from lesion. These alterations were not present at the earlier post-operative time points.

In conclusion, this study shows that BDNF levels are reduced in a rat model of cholinergic degeneration and suggests that these alterations may occur at later stages. In addition, a positive correlation between serum and parietal cortex changes is observed. Even if the cause for the relationship between BDNF in serum and this brain region is unknown, these data may help to elucidate the significance of peripheral and central BDNF changes in brain pathological conditions.

1. Introduction

The neurotrophin brain-derived neurotrophic factor (BDNF) has been extensively studied because of its role in survival, differentiation and function of neurons undergoing degeneration in pathological conditions, such as cholinergic neurons in Alzheimer's disease (AD) (Mattson et al., 2004). This line of investigation was supported by a consistent number of experimental evidences. Studies in serum and post-mortem brains of AD patients have documented alterations in BDNF expression (Connor et al., 1997; Durany et al., 2000; Peng et al., 2005) while in animal models BDNF has generated interest as a potential neuroprotective agent of cholinergic neurons which undergo to degeneration and death during AD course (Xuan et al., 2008; Nagahara et al., 2009). However, despite these numerous studies, the role of BDNF in these events is still matter of debate because results are often in opposite direction.

The reasons for such different results are not well known. There is evidence that different stages of AD may be characterized by different directions in BDNF changes (Laske et al., 2006, 2007; Yasutake et al., 2006). Another puzzling factor is represented by pharmacological treatments known to cause alterations of BDNF peripheral levels, such as acetylcholinesterase inhibitors (AchEI) or antidepressants when prescribed. A pivotal issue would be to verify whether brain and serum BDNF changes are interconnected as well as the possibility that different stages of cholinergic degeneration are characterized by different changes in BDNF brain and serum levels.

Consequently, in order to investigate the relationship between brain and serum BDNF levels at different stages of cholinergic degeneration, in the present study we used a rat model based on intracerebroventricular (i.c.v.) injections of 192 IgG-Saporin (Heckers et al., 1994) and measured the brain and serum BDNF levels by enzyme-linked immunosorbent assay (ELISA) at 3, 7 and 15 days from immunotoxin injection.

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2. Materials and methods

2.1. Animals

Adult male Wistar rats (250–300 g; Harlan, Italy) were used in the study. Rats were kept under standard conditions with food and water *ad libitum* on a 12/12 h dark/light cycle. Animals were maintained according to the guidelines for ethical conduct developed by the European Communities Council Directive of November 24, 1986 (86/609/EEC). All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Surgery

Rats were assigned to two experimental groups: Saporin and Phosphate-Buffered Saline (PBS) treated rats. All rats were i.p. anesthetized with Zoletil 100 (Tiletamine and Zolazepam: 50 mg/kg – Virbac s.r.l., Milan, Italy) and Rompun (Xylazine: 10 mg/kg – Bayer s.p.a., Milan, Italy). In one group, immunotoxin 192 IgG-Saporin (Chemicon International Inc., Harrow, UK) was bilaterally injected into lateral ventricles through a 10-μL Hamilton syringe at the following coordinates: AP: −0.8 mm (from the bregma); ML: ±1.4 mm (from the midline); DV: −3.5 mm (from the dura). 2 μL/side of immunotoxin 192 IgG-Saporin diluted in PBS (2 μg/μL) was injected at a rate of 1 μL/min. At the end of administration, the needle was left in situ for 5 min. The same volume of PBS, but containing no Saporin, was injected in the remaining rats.

2.3. Tissue dissection

At 3, 7, and 15 days from surgery, rats of Saporin and PBS groups (4 animals/group) were decapitated (starting at 10.00 A.M.) and the brains were quickly removed and dissected on ice using a binocular dissection microscope. Frontal cortex, hippocampus, parietal cortex and striatum were collected according to Glowinski and Iversen's method (1966). All brain regions were extracted in 1 ml extraction buffer/100 mg tissue. Brain tissue samples were homogenized in an ice-cold lysis buffer containing 137 mM NaCl, 20 mM Tris–HCl (pH 8.0), 1% NP40, 10% glycerol, 1 mM PMSF 10 μg/ml aprotinin, 1 μg/ml leupetin and 0.5 mM sodium vanadate. The tissue homogenate solutions were centrifuged with 14,000*g* for 25 min at 4 C. The supernatants were collected and used for quantification of BDNF.

2.4. Blood sampling

Blood samples were collected into sampling tubes that were centrifuged within 20 min at 2000*g* for 20 min. Serum was then aliquoted and stored at −80 °C until analysis.

2.5. Choline acetyl transferase (ChAT) assay

To validate our experimental model in term of cholinergic damage in Saporin-treated animals we measured ChAT activity in each hemi-hippocampus and hemi-frontal cortex of rats in Saporin and PBS groups. The procedure used is the same as that reported in other papers (Gelfo et al., 2011).

2.6. BDNF determination by enzyme-linked immunosorbent assay (ELISA)

Concentrations of BDNF protein in serum and brain were assessed using a two-site enzyme immunoassay kit (Promega, USA) following the manufacturer's instructions. BDNF concentrations were determined from the regression line for the standard (ranging from 7.8 to 500 pg/ml-purified mouse BDNF) incubated under similar conditions in each assay. For each assay kit, the cross-reactivity with other related neurotrophins was less than 3%. BDNF concentration was expressed as pg/g wet weight in brain and pg/ml in serum. All assays were performed in triplicate.

2.7. Statistical analysis

The data were analyzed by means of one-way analyses of variance (ANOVA) considering treatment with Saporin and PBS as variables. Correlation between BDNF brain and serum levels was evaluated with Spearman's rank correlation test. *P*-values $\leqslant 0.05$ were considered statistically significant.

3. Results

3.1. Effect of Saporin on body weight and ChAT activity

Saporin did not induce significant changes in body weight. The weights of the brain regions examined were also comparable between PBS and Saporin-treated rats (not shown). Analysis of ChAT activity at 3, 7 and 15 days from lesion confirmed a reduction in enzymatic activity in both hippocampus and frontal cortex in Saporin-treated animals, already observed in previous studies performed in our laboratory (Gelfo et al., 2011).

3.2. BDNF brain levels in Saporin and PBS treated rats

BDNF brain levels are shown in Fig. 1. In frontal cortex, hippocampus and striatum no significant effects following Saporin injection were noted at 3, 7, and 15 days from the lesion. In the parietal cortex, Saporin injection did not significantly alter BDNF levels at 3 and 7 days from the lesion. However, at 15 days from the lesion BDNF levels were significantly reduced ($F = 10.73$; $p < 0.05$) in Saporin-treated rats, as compared to PBS-treated rats (Fig. 1).

3.3. BDNF serum levels in Saporin and PBS treated rats

BDNF serum levels are shown in Fig. 2. Saporin injection did not induce significant effect at 3 and 7 days from lesion. However, at 15 days from lesion BDNF serum levels were significantly lower in Saporin-treated rats as compared to PBS-treated rats ($F = 6.70$; $p < 0.05$) (Fig. 2).

3.4. Correlation between brain and serum BDNF levels

A significant positive correlation was found between parietal cortex and serum BDNF levels at 15 days from lesion ($R = 0.786$; $p < 0.05$). At 3 and 7 days from lesion no significant correlation between parietal cortex and serum was observed. In the other brain regions examined no other significant correlations were found at 3, 7 and 15 days from lesion.

4. Discussion

In the present study we used a well established animal model of cholinergic degeneration (i.c.v. injections of the selective immunotoxin 192 IgG-Saporin) to verify whether brain and serum BDNF changes occur at same or different times from immunotoxin lesion and whether these changes are in the same or opposite direction. We found that BDNF levels were reduced in parietal cortex and serum of Saporin-treated rats at 15 days from lesion. Moreover, a positive correlation between serum and parietal cortex was also observed at 15 days. These alterations were not present at the