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Neurotrophins in murine viscera: a dynamic pattern from birth to adulthood

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Abstract

There is growing evidence that target-derived neurotrophins regulate the function of visceral neurons after birth. However, the postnatal profile of neurotrophin supply from internal organs is poorly described. In this study, we compared neurotrophin concentrations in lysates of murine peripheral target tissues (lung, heart, liver, colon, spleen, thymus, kidney and urinary bladder) at different time points after birth. In most organs, there was a decrease of neurotrophin concentrations in the first weeks after birth. In contrast, there were characteristic increases of specific neurotrophins during adolescence or adulthood. These increases were found for nerve growth factor (NGF) in the heart, thymus, kidney and liver, for brain-derived neurotrophic factor (BDNF) in the lung, and for neurotrophin-3 (NT-3) in the colon. In conclusion, we show that neurotrophins display a very differential and dynamic profile in internal organs after birth.

Keywords: Neurotrophins; Viscera; Postnatal development

1. Introduction

The neurotrophins nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) are members of a family of structurally related proteins regulating neuronal survival, development, function and plasticity (Lewin and Barde, 1996). It has been well established that neurotrophins play a crucial role for the survival and differentiation of visceral neurons during development (Snider, 1994; Huang and Reichardt, 2001). The extent to which neurotrophins are involved in the physiology of mature peripheral neurons is less clear. Neurotrophins are expressed in visceral targets of adult rodents and humans (Katoh-Semba et al., 1989; Timmusk et al., 1993; Zhou and Rush, 1993; Katoh-Semba et al., 1996; Yamamoto et al., 1996; Lommatzsch et al., 1999), and retrogradely transported in adult visceral sensory and motor neurons (Helke et al., 1998). Notably, a variety of mature neurons acquire neurotrophins from the periphery which have not been dependent on these neurotrophins for survival during development. In turn, some neuronal subpopulations do not acquire neurotrophins in adulthood which have been dependent on these neurotrophins during development (Helke et al., 1998). Therefore, the functions of neurotrophins in sensory and motor neurons are likely to change during ontogenesis.

The issue whether postnatal neurons still require neurotrophins for survival remains controversial (Tafreshi

Abbreviations: NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; NT-3, neurotrophin-3; ELISA, enzyme-linked immunosorbent assay; Trk, tropomyosin kinase

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et al., 1998; Mendell, 1999; Ruberti et al., 2000; Stucky et al., 2002). In contrast, there is growing evidence that neurotrophins play a role in regulating plasticity and function of adult neurons (McAllister et al., 1999). Neurotrophins influence neuropeptide expression, synaptic plasticity and excitability of adult sensory and motor neurons (Lindsay and Harmar, 1989; Dmitrieva and McMahon, 1996; Causing et al., 1997; Gonzalez and Collins, 1997; Romero et al., 2000). Given the retrograde transport of neurotrophins in adult visceral afferents and efferents, a target-derived modulation of neuronal function appears likely (Zhou et al., 1997). Although changes of visceral neurotrophin levels have been described in pathological conditions (Dmitrieva et al., 1997; Oddiah et al., 1998; Braun et al., 1999), there is little data on the natural course of neurotrophin supply from internal organs after birth (Vizzard et al., 2000; Lipps, 2002). These information, however, are needed to further understand the significance of altered neurotrophin levels described in pathological conditions of adult internal organs. It was the aim of this study, therefore, to investigate systematically the postnatal profile of neurotrophin concentrations in visceral tissues.

2. Experimental procedures

Three-month-old pairs of Balb/c mice were obtained from Harlan-Winkelmann (Borchen, Germany) and bred under controlled conditions. Twenty female pups from the litters obtained were divided into four groups. Animals of group 1 ($n = 5$) were killed 1 day after birth by decapitation. The other groups were maintained for 21 days (group 2, $n = 5$), 120 days (group 3, $n = 5$) and 365 days (group 4, $n = 5$) after birth under controlled conditions, and then killed by cervical dislocation. In a seperate experiment, 21 days ($n = 3$) and 120 days ($n = 3$) old female Balb/c mice were examined for neurotrophin recovery experiments. Animal treatment protocols were approved by the animal care committee of the Charité (Humboldt University of Berlin, Germany), and the experiments carried out in accordance with the European Communities Council Directive (86/609/EEC). Preparation of tissue lysates was performed as described before (Lommatzsch et al., 1999). Total protein content in tissue lysates was measured using the detergent-compatible BCA protein assay (Pierce, Rockford, USA). Neurotrophin protein levels (NGF, BDNF and NT-3) in lysates were measured in duplicate using commercial ELISA kits according to the manufacturer's instructions (DuoSet, R&D Systems, Minneapolis, USA). The detection limit was 8–16 pg/ml for each neurotrophin. Neurotrophin-4 (NT-4) measurements were not incorporated into this paper, since there are concerns about the specificity of NT-4 measurements in terms of our tissue extraction method (Zhang et al., 2000). Concentrations of neurotrophins were calculated as nanograms of neurotrophin per gram of total protein. For specificity control, 2000 pg/ml of each recombinant neurotrophin were put on ELISA plates of the other neurotrophins. In every neurotrophin ELISA, other neurotrophins remained below the detection limit, indicating no cross-reactivity between the three measured neurotrophins (<1%). For neurotrophin recovery experiments, solutions containing 5 mg bovine serum albumine (the protein used for dissolving neurotrophin standards in ELISA measurements) per millilitre of phospate buffered saline (PBS) were used as a control solution. Tissue lysates were diluted to the same protein concentration (5 mg total protein per millilitre of PBS). Control and tissue lysate solutions were mixed 1:1 with PBS containing 1000 pg neurotrophin per millilitre, or with PBS alone. Neurotrophin recovery was calculated by subtracting the amount of neurotrophin measured in samples without exogenous neurotrophin from corresponding samples with exogenous neurotrophin. Neurotrophin recovery in lysates is expressed as percentage of recovery in control solutions. Results are presented as mean values ± standard deviation (S.D.). Means were compared by using analysis of variance (ANOVA with SPSS, Chicago, IL). P-values < 0.05 were regarded as significant differences.

3. Results

3.1. Neurotrophin recovery

To evaluate whether neurotrophins may be bound to other molecules in lysates of murine organs, we measured the recovery of exogenous BDNF, NGF and NT-3 in organ

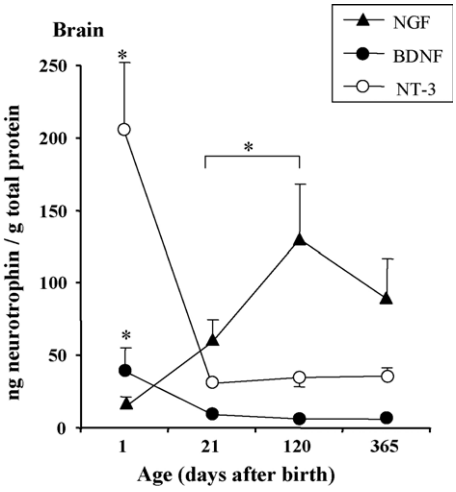

Fig. 1. Postnatal neurotrophin profile in total brain lysates. Total brains were taken from female Balb/c mice at different ages: 1, 21, 120 and 365 days old ($n = 5$ in each cohort). Concentrations are displayed as nanograms of neurotrophin per gram of total protein. Shown are arithmetic means ± each standard deviation. Brackets with an asterisk show significant differences between two time points after birth. Single asterisks (*) mark time points with significantly higher neurotrophin levels than at all other time points examined.