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Research Report

Brain-derived neurotrophic factor (BDNF) and polysialylated-neural cell adhesion molecule (PSA-NCAM): codistribution in the human brainstem precerebellar nuclei from prenatal to adult age

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ABSTRACT

Occurrence and distribution of the neurotrophin brain-derived neurotrophic factor (BDNF) and polysialylated-neural cell adhesion molecule (PSA-NCAM), a neuroplasticity marker known to modulate BDNF signalling, were examined by immunohistochemistry in the human brainstem precerebellar nuclei at prenatal, perinatal and adult age. Western blot analysis performed in human brainstem showed for both molecules a single protein band compatible with the molecular weight of the dimeric form of mature BDNF and with that of PSA-NCAM. Detectability of both molecules up to 72 h post-mortem was also assessed in rat brain. In neuronal perikarya, BDNF-like immunoreactivity (LI) appeared as intracytoplasmic granules, whereas PSA-NCAM-LI appeared mostly as peripheral staining, indicative of membrane labelling; immunoreactivity to both substances also labelled nerve fibres and terminals. BDNF- and PSA-NCAM-LI occurred in the external cuneate nucleus, perihypoglossal nuclei, inferior olive complex, arcuate nucleus, lateral reticular formation, vestibular nuclei, pontine reticulotegmental and paramedian reticular nuclei, and pontine basilar nuclei. With few exceptions, for both substances the distribution pattern detected at prenatal age persisted later on, though the immunoreactivity appeared often higher in pre- and full-term newborns than in adult specimens. The results obtained suggest that BDNF operates in the development, maturation, maintenance and plasticity of human brainstem precerebellar neuronal systems. They also imply a multiple origin for the BDNF-LI of the human cerebellum. The codistribution of BDNF- and PSA-NCAM-LI in analyzed regions suggests that PSA-NCAM may modulate the functional interaction between BDNF and its high and low affinity receptors, an issue worth further analysis, particularly in view of the possible clinical significance of neuronal trophism in cerebellar neurodegenerative disorders.

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1. Introduction

Bidirectional cellular interactions with afferent neurons control the establishment and maintenance of cerebellar circuitry (Lohof et al., 2005; Madalosso et al., 2005; Letellier et al., 2007; Azizi, 2007). The brainstem precerebellar nuclei represent the source for a large part of the afferent input to the cerebellum (Voogd, 2004; Ruigrok, 2004) and several substances act in the interplay of instructive signals in their development, preservation and repair (Sherrard and Bower, 2001, 2002; de Diego et al., 2002; Bloch-Gallego et al., 2005; Dixon and Sherrard, 2006; Marcos et al., 2009). In particular, studies in rodents show that the neurotrophin brain-derived neurotrophic factor (BDNF) is involved in the development of olivary (Rocamora et al., 1993; Sherrard and Bower, 2001, 2002; Dixon and Sherrard, 2006) and pontocerebellar neurons (Rabacchi et al., 1999), and in postnatal maturation and regulation of vestibular nuclei (Montcouquiol et al., 2000). Moreover, BDNF stimulates neuronal plasticity of axotomized olivary neurons in both neonatal and adult rats (Sherrard and Bower, 2001, 2002; Dixon and Sherrard, 2006; Willson et al., 2008) and contributes to vestibular compensation after unilateral labyrinthectomy (Li et al., 2001a,b).

BDNF acts upon binding to the specific tyrosine-kinase receptor trkB and to the common neurotrophin receptor p75 (Kaplan and Miller, 2000). It is known that the polysialylated form of the neural cell adhesion molecule (PSA-NCAM) (Rutishauser, 2008) can affect these ligand–receptor interactions. In fact, PSA-NCAM has been shown to facilitate the interaction of BDNF with trkB (Muller et al., 2000; Vutskits et al., 2001) and to regulate the expression of p75 (Gascon et al., 2007), thereby modulating the neuronal sensitivity to the trophin.

The relevant role of BDNF activity in the correct development and function of precerebellar nuclei contrasts with the paucity of information regarding the occurrence and functional significance of this molecule in the human precerebellar system. Indeed, data regarding the expression of BDNF in the human nervous system (Phillips et al., 1990; Murer et al., 1999, 2001; Webster et al., 2002, 2006; Quartu et al., 2003; Wong et al., 2009), and all the more those describing its changes with age (Webster et al., 2002, 2006; Quartu et al., 2003; Wong et al., 2009; Tang et al., 2010), are limited. In a similar way, data on PSA-NCAM occurrence in the human nervous system are fragmentary, mainly related to specific forebrain regions (Barbeau et al., 1996; Mikkonen et al., 1998; Ní Dhúill et al., 1999; Arellano et al., 2002; Ulfig and Chan, 2004; Jakovcevski et al., 2007; Varea et al., 2007; Quartu et al., 2008) and peripheral nerve (Roche et al., 1997), and not focussed on the possible relation to the BDNF trophic machinery. We reported previously that extensive BDNF-labelled nerve fibre systems, likely of external origin, occur in the human cerebellum and that their density and territory of distribution increase with age from prenatal to early postnatal and adult life (Quartu et al., 2003). With the aim of gaining information relevant to the involvement of the neurotrophin in the neuronal viability, circuitry organization and plasticity in precerebellar nuclei, we examined the normal distribution of BDNF in the human brainstem precerebellar system by immunohistochemistry. In parallel, we analyzed the occurrence of PSA-NCAM in the same areas. In order to acquire data relevant to the temporal profile of BDNF and PSA-NCAM expression, as far as compatible with the obvious limitations of human tissue sampling, we extended the analysis to the widest possible range of age, from prenatal to adult life. The immunodetectability of BDNF and PSA-NCAM has also been examined by western blot in human brainstem regions and, in order to test their post-mortem stability, in rat hippocampus sampled up to 72 h after sacrifice.

2. Results

2.1. *Western blot*

In samples of human medulla oblongata and mesencephalic tegmentum, the antiserum against BDNF revealed a single band at approximately 27 kDa at all ages examined (Fig. 1A, lanes 1–4). When challenged with the recombinant human (rh) BDNF, the antiserum labelled two bands, a thicker one at a level corresponding to the molecular weight (mw) of the monomeric form of the neurotrophin (about 14 kDa), and a thinner one corresponding to the mw of the dimeric form of BDNF (about 27 kDa) (Fig. 1A, lane 5). Software analysis confirmed the mw equivalence of the bands detected in human samples and that at higher mw of the rhBDNF. The BDNF antiserum did not cross-react with the rh nerve growth factor (rhNGF) (Fig. 1A, lane 6). Control immunostaining carried out with the BDNF antiserum preabsorbed with 200 ng of rhBDNF revealed no staining in tissue homogenates (Fig. 1B lanes 1–4), totally abolished immunolabelling of rhBDNF dimeric form and greatly reduced that of its monomeric form (Fig. 1B, lane 5). The latter was totally prevented by pre-absorption of the antiserum with 600 ng of rhBDNF (not shown). As in human brainstem, a single BDNF-like immunoreactive band at about 27 kDa was detected in rat hippocampus homogenates and, though growing thinner at 72 h post-mortem, it remained obvious up to that time interval (Fig. 1D).

In samples of human medulla oblongata, the anti-PSA-NCAM antibody labelled a single band (Fig. 1F, lanes 1–3), whose level matches the expected mw (Dubois et al., 1994; Quartu et al., 2008). By contrast and in agreement with immunohistochemical results (below), no stained bands were detected in samples of human mesencephalic tissue (Fig. 1F, lane 4). Control immunostaining carried out with the anti-PSA-NCAM antibody preadsorbed with 5 mg of colominic acid resulted in no staining (Fig. 1G). The stability of PSA-NCAM-like immunoreactive band up to 72 h post-mortem was shown in rat hippocampal homogenates (Fig. 1I).

2.2. *Immunohistochemistry*

The BDNF-like immunoreactivity (LI) appeared as intracytoplasmic granules of different size and density in the perikaryon and proximal processes of neuronal cells, and as thread- and dot-like elements, interpreted as nerve fibres and terminals. The PSA-NCAM-LI appeared mostly as a peripheral staining in neuronal cell bodies and their proximal processes, indicative of membrane labelling, and as varicose and smooth filaments and punctate elements, interpreted as nerve fibres and terminals,