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Int. J. Devl Neuroscience 23 (2005) 425–438

INTERNATIONAL JOURNAL of DEVELOPMENTAL NEUROSCIENCE

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Ret, GFRalpha-1, GFRalpha-2 and GFRalpha-3 receptors in the human hippocampus and fascia dentata

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Received 14 February 2005; received in revised form 24 March 2005; accepted 3 May 2005

Abstract

The immunohistochemical occurrence and localization of the receptor components of the glial cell line-derived neurotrophic factor (GDNF) family ligands, the Ret receptor tyrosine kinase and GDNF family receptor (GFR) alpha-1 to -3, is described in the human post-mortem hippocampal formation at pre- and full-term newborn, and adult age. Two different antibodies for each of the four-receptor molecules were used. Western blot analysis indicates that the availability of GFRalpha receptor proteins may vary with age and post-mortem delay. The immunohistochemical detectability of GFRalpha-1, GFRalpha-2, GFRalpha-3 and Ret receptor molecules is shown in the rat up to 72 h post-mortem. In the human specimens, labelled neuronal perikarya were detectable for each receptor protein at all examined ages, with prevalent localization in the pyramidal layer of the Ammon's horn and hilus and granular layer of the fascia dentata. In the adult subjects, abundant punctate-like structures were also present. Labelled glial elements were identifiable. Comparison of the pattern of immunoreactive elements among young and adult subjects suggests that the intracellular distribution of the GDNF family ligands may vary between pre- and perinatal life and adult age. The results obtained suggest the involvement of the Ret and GFRalpha receptors signalling in processes subserving both the organization of this cortical region during development and the functional activity and maintenance of the mature hippocampal neurons.

Keywords: Ret receptor tyrosine kinase; GFRalpha receptors; GDNF-family neurotrophic factors; Hippocampal formation; Man; Western blot; Immunohistochemistry; Post-mortem detectability

1. Introduction

The receptor complex for the glial cell line-derived neurotrophic factor (GDNF) family ligands comprises a common transmembrane signalling component, the Ret receptor tyrosine kinase (Durbec et al., 1996; Treanor et al., 1996; Trupp et al., 1996) and a ligand-binding receptor, which belongs to a family of glycosyl phosphatidylinositol (GPI)-anchored membrane proteins, called GDNF family receptor alpha (GFRalpha). Four distinct GFRalpha receptors have been described, GFRalpha-1 to GFRalpha-4, which mediate the influence of the GDNF family trophins on the development, differentiation, and maintenance of mature phenotype of distinct and partially overlapping central and peripheral neuronal populations (see Airaksinen and Saarma, 2002). Each GFRalpha receptor interacts preferentially with one of the GDNF family ligands. Thus, GDNF, the earliest discovered factor of the family, binds preferentially to GFRalpha-1, neurturin (NTN) to GFRalpha-2, artemin (ART) to GFRalpha-3, and persephin (PSP) to GFRalpha-4 (Jing et al., 1996, 1997; Baloh et al., 1997, 1998; Klein et al., 1997; Enokido et al., 1998). Once GDNF family ligands bind to their relevant GFRalpha receptor, the ensuing ligand/receptor complex can interact with Ret and induce its homodimerization, autophosphorylation, and

Abbreviations: CA, Cornus Ammonis; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GDNF, glial cell line-derived neurotrophic factor; GFAP, glial fibrillary acidic protein; GFRalpha, GDNF family receptor alpha; NTN, neurturin; ART, artemin; PSP, persephin; mw, molecular weight

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0736-5748/$30.00
doi:10.1016/j.ijdevneu.2005.05.003

subsequent activation of various cytoplasmic signalling pathways (see Airaksinen et al., 1999). Ret and GFRalpha receptors have been widely studied by Northern blot analysis and in situ hybridization histochemistry in the rat and mouse brain and their expression has been found consistent with areas of the brain where neurons responsive to the GDNF family ligands are located (Treanor et al., 1996; Trupp et al., 1996; Trupp et al., 1997; Nosrat et al., 1997; Suvanto et al., 1997; Sanicola et al., 1997; Widenfalk et al., 1997; Glazner et al., 1998; Golden et al., 1998; Masure et al., 1998, 1999; Naveilhan et al., 1998; Worby et al., 1998; Yu et al., 1998; Burazin and Gundlach, 1999). Experimental evidence indicates that GDNF family trophic factors play neuroprotective and neuroreparative roles after brain injury and in animal models of neurodegeneration (Tseng et al., 1998; Kitagawa et al., 1999; Kokaia et al., 1999; Miyazaki et al., 1999, 2001, 2002; Rosenblad et al., 1999, 2000; Perez-Navarro et al., 2000; Tsai et al., 2000; Ardvinsson et al., 2001; Gratacos et al., 2001; Sarabi et al., 2001; Marco et al., 2002a,b; Tomac et al., 2002), implicating a potential for them as therapeutic agents. Though the expression of GFRalpha receptors appears not to be matched by that of Ret receptors in some brain regions, transcripts for Ret and one of the GFRalpha receptors are usually co-expressed (Trupp et al., 1997; Glazner et al., 1998; Golden et al., 1998; Masure et al., 1998; Worby et al., 1998; Burazin and Gundlach, 1999).

Ret and GFRalpha receptors mRNAs are expressed in the hippocampus of rodents during development (Nosrat et al., 1997; Lenhard and Suter-Crazzolara, 1998; Golden et al., 1999) and in adult life (Nosrat et al., 1997; Trupp et al., 1997; Glazner et al., 1998; Masure et al., 1998; Reeben et al., 1998; Burazin and Gundlach, 1999; Golden et al., 1999). Quantitative data on the expression of Ret, GFRalpha-1, and GFRalpha-2 show that their levels, low throughout life, are fairly high between postnatal day 0 and 14, a crucial period for hippocampal neuronal migration and synaptogenesis (Lenhard and Suter-Crazzolara, 1998). Moreover, their expression is differentially and dynamically modulated in the adult following insults such as epileptic seizures (Trupp et al., 1997; Reeben et al., 1998; Kokaia et al., 1999; Chen et al., 2001; Gao et al., 2003) and forebrain ischemia (Kokaia et al., 1999; Ardvinsson et al., 2001; Sarabi et al., 2001, 2003; Miyazaki et al., 2002). Data on GFRalpha-2 knockout mice indicate that signalling through this receptor affects the seizure susceptibility of hippocampal neurons (Nanobashvili et al., 2000, 2003). Functional studies have also shown the efficacy of GDNF (Martin et al., 1995; Bonde et al., 2000; Cheng et al., 2004) and ART (Bonde et al., 2000) in protecting hippocampal neurons from excitotoxic insults. Moreover, the exogenous administration of GDNF can prevent epileptic seizures after kainic acid administration (Martin et al., 1995), modulates kindling and activation-induced mossy fibres sprouting (Li et al., 2000), and provides protection against delayed apoptotic neuronal death induced by ischemia (Miyazaki et al., 1999). To our knowledge, studies aimed at determining the tissue localization of Ret and GFRalpha molecules in the hippocampus are limited to GFRalpha-1 in the rat (Matsuo et al., 2000; Sarabi et al., 2001). The potential relevance of GDNF family ligands and their receptors in the normal activity and in the pathological events involving the hippocampus contrasts with the absence of information concerning the involvement of these molecules in the trophism of the human hippocampal formation. Our previous studies on the tissue localization of GDNF mRNA and protein (Serra et al., 2002a,b) and NTN, PSP, and ART in the human hippocampal formation from prenatal to adult age (Quartu et al., 2005) support their involvement in the development, differentiation, and maintenance of the human hippocampal circuitry. This investigation examines the immunohistochemical occurrence of Ret and GFRalpha-1, GFRalpha-2, and GFRalpha-3 in the human post-mortem hippocampus and fascia dentata at life stages spanning from prenatal age to adulthood.

2. Materials and methods

Autopsy specimens of human hippocampal formation were obtained from subjects of different ages, with no history of neuropathology (Table 1). The sampling and handling of human specimens has been conducted under the appropriate ethical safeguards and protocols of the Section of Pathological Anatomy, Department of Cytomorphology, University of Cagliari. Sixteen male adult Sprague-Dawley rats were sacrificed by decapitation and their head kept at 4 °C for 0, 24, 48, and 72 h post-mortem interval. At each time step four brains were dissected out, two for western blot analysis and two for immunohistochemistry.

2.1. Western blot

Human autoptic tissue blocks of hippocampus (Ammon's horn) and fascia dentata from two pre-term (cases 1 and 5), a full-term (case 7) and five adult subjects (cases 12, 13, 15, 19 and 20) and the hippocampus of Sprague-Dawley adult rats were collected and stored at −80 °C until required. Tissue homogenates were prepared by homogenization in 10 volumes of water containing 2% sodium dodecyl sulphate (SDS). Protein concentrations were determined using the Lowry method of protein assay (Lowry et al., 1951) with bovine serum albumin as standard. Proteins for each tissue homogenate (40 μg) diluted 1:1 in loading buffer were heated to 95 °C for 3 min and separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using 7.5–12.5% (w/v) polyacrylamide resolving gel. Internal molecular weight standards (Kaleidoscope Prestained Standards, Bio-Rad, Hercules, CA, USA) were run in parallel. Two gels at a time were run for Coomassie staining and immunoblotting, respectively. Proteins for immunoblotting were electrophoretically transferred on a poly-