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Research report

Effect of GDNF on differentiation of cultured ventral mesencephalic dopaminergic and non-dopaminergic calretinin-expressing neurons

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Abstract

Glial cell line-derived neurotrophic factor (GDNF) is a potent survival factor for ventral mesencephalic (VM) dopaminergic neurons. Subpopulations of dopaminergic and non-dopaminergic VM neurons express the calcium-binding proteins calbindin (CB) and calretinin (CR). Characterization of the actions of GDNF on distinct subpopulations of VM cells is of great importance for its potential use as a therapeutic molecule and for understanding its role in neuronal development. The present study investigated the effects of GDNF on the survival and morphological differentiation of dopaminergic and non-dopaminergic neurons in primary cultures of embryonic day (E) 18 rat VM. As expected from our results obtained using E14 VM cells, GDNF significantly increased the morphological complexity of E18 CB-immunoreractive (CB-ir), tyrosine hydroxylase (TH)-ir, and CR-ir neurons and also the densities of CB-ir and TH-ir neurons. Interestingly, densities of E18 CR-ir neurons, contrarily to our previous observations on E14 CR-ir neurons, were significantly higher after GDNF treatment (by 1.5-fold). Colocalization analyses demonstrated that GDNF increased the densitiy of dopaminergic neurons expressing CR (TH+/CR+/CB−), while no significant effects were observed for TH−/CR+/CB− cell densities. In contrast, we found that GDNF significantly increased the total fiber length (2-fold), number of primary neurites (1.4-fold), number of branching points (2.5-fold), and the size of neurite field per neuron (1.8-fold) of the non-dopaminergic CR-expressing neurons (TH−/CR+/CB−). These cells were identified as GABA-expressing neurons. In conclusion, our findings recognize GDNF as a potent differentiation factor for the development of VM dopaminergic and non-dopaminergic CR-expressing neurons.

Theme: Development and regeneration
Topic: Neurotrophic factors: biological effects

Keywords: Glial cell line-derived neurotrophic factor; Tyrosine hydroxylase; Calbindin; Calretinin; Cell morphology; Ventral mesencephalon

1. Introduction

Glial cell line-derived neurotrophic factor (GDNF) family belongs to a distant branch of the transforming growth factor-β superfamily [11,35], which comprises neurturin [31], persephin [41], and artemin [8,36,52]. GDNF family ligands (GFLs) have been described to be potent survival factors for neurons of the central and peripheral nervous systems [1,11,19,25,26,32,35,41,65,67]. The cellular responses to GFLs are generally assumed to be mediated by a multicomponent receptor complex composed of the common RET membrane-bound tyrosine kinase [15,28,61] and members of a glycosyl phosphatidylinositol (GPI)-anchored family of receptors named GDNF family receptor α (GFR-α). In the light of recent findings, however, a more complex biology of GDNF signaling has to be considered [9,45,46,48,55,60]. GFRα1 is the preferred receptor for GDNF and Sarabi and co-workers demonstrated that GFRα1 is expressed in dopaminergic and non-

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dopaminergic midbrain neurons [54]. GDNF is expressed throughout the rat CNS with relatively high levels in the developing striatum [13,58], and low levels in the ventral mesencephalon [13,56].

A spatial and compartmental organization of rat VM dopaminergic neurons has previously been described [22,38]. Dopaminergic cells are organized in two bands, one rostrodorsal corresponding to the substantia nigra pars compacta (SNC) and one caudoventral corresponding to the SN pars reticulata (SNR). Ontogenically, the ventral dopaminergic cells are born between E12–15 and distribute in the SNR and in the most ventral layer of the SNC [16,21]. The dorsal part contain dopaminergic cells generated between E17 and the first postnatal week and occupies the ventral tegmental area and the dorsal segment of the SNC [2].

Calretinin (CR) is a calcium-binding protein expressed in several neuronal populations in the CNS [4,6,7,12,24,49]. The ventral mesencephalon (VM) contains cell populations expressing CR as well as the other calcium-binding proteins that share the structural EF-hand motif [7], calbindin-D28k (CB), and parvalbumin (PV) [2,27,33,51]. The SNC can be divided into subcompartments based on the distribution of dopaminergic cell populations expressing the calcium-binding proteins CR and/or CB [22,43,51]. CR and CB show a distinct pattern of expression during development. The CR immunoreactive cells appear relatively early in the rostral SN and are thereafter distributed throughout the SNC most prominently in the ventral parts. CB immunoreactive cells develop later and are restricted to the dorsal SNC [2]. The functional significance of this compartmentalization is still not known. Calcium-binding proteins are believed to regulate cellular activities such as cell proliferation, migration, and differentiation by suppressing or buffering intracellular calcium [2,7,23]. Interestingly, a role of calretinin as calcium regulator until neurotrophins take over this function has been supposed in dorsal root rat ganglia cells [3].

We have previously shown in cultures derived from E14 rat fetuses that GDNF promoted survival of mesencephalic CB-ir and TH-ir but not CR-ir cells and supported morphological differentiation of all three types of neurons. However, in this study, we did not specifically distinguish between the different subpopulations. In the present study, we aimed at investigating the effects of GDNF on more differentiated ventral mesencephalic neurons, i.e., from E18 old rat embryos. At this developmental stage, a striatonigral projection from neurons localized in striatal patches has been established [34]. Ontogeny of TH gene expression in the ventral mesencephalon has been reported to exhibit two phases, an early (E13–E15) and a later phase (E18–E21) when TH mRNA expressing neurons reached their final anatomical position within the mesencephalic DA complex [57]. Moreover, a rapid development of CR in the developing SN between E18 and postnatal day three was reported [2].

The neuroprotective and the potential neuroregenerative effects of GDNF noticed on dopaminergic neurons in animal models of Parkinson's disease (PD) strongly hinted at its potential as a therapeutic agent for PD. The importance of an elaborate knowledge of actions of GDNF is further supported by a recent report showing that the presence of GDNF induced the expression of TH in late developmental stages of cultured neural progenitor cells. Particularly, based on the authors' suggestion that using appropriate factor application may provide a robust tool to interfere with final cell fate specification of neural progenitor cells [59].

The aim of the present study was hence to provide a comprehensive description of the effects of GDNF on the survival and morphological differentiation of cultured E18 VM cells with special emphasis on the non-dopaminergic CR-expressing neurons. Parts of the results have been published previously in abstract form [66].

2. Materials and methods

2.1. Cell cultures and growth factor treatment

Rat fetuses staged at embryonic day (E) 18 (E0 = day of vaginal plug) were removed from deeply anesthetized (Nembutal; 100 mg/kg body weight) Sprague–Dawley rats (BRL Biological Research, Füllinsdorf, Switzerland). Ventral mesencephalic areas were dissected in cold Dulbecco's modified Eagle medium (DMEM; GIBCO), and cell suspensions were prepared according to previously published procedures [29,30,65]. 150,000 cells/well were seeded into 24-well plates (TIPP, Switzerland) with 0.5 ml of growth medium/well (see below). Culture wells contained glass coverslips with a diameter of 12 mm (Assistent, Germany) that were previously coated overnight in the incubator at 37 °C with a solution of 0.1 mg/ml poly-L-lysine (Sigma) in 0.15 M sodium borate buffer, pH 8.3. The wells were washed three times with sterile PBS and once with serum containing medium before adding the medium.

Cultures were grown in medium containing 55% DMEM (GIBCO), 32.5% Hank's balanced salt solution (GIBCO), 1.5% glucose, 10% fetal calf serum (GIBCO), and 1% 0.01 M HEPES. Antibiotics (GIBCO) were present for the first 3 days. Cells were incubated at 37 °C in a 95% air/5% CO_2 humidified atmosphere. The medium was changed 24 h after plating and subsequently every other day. Cultures were randomly assigned to the GDNF group and to the control group. GDNF (10 ng/ml) (Promega) was added at day 0 and then at each medium change. Control cultures received medium with no trophic factor added. Seven days after plating, the cultures were processed for histology.

2.2. Immunohistochemistry and cell density measurements for tyrosine hydroxylase, calretinin-, and calbindin-expressing neurons

Cultures were washed two times in 0.1 M phosphate buffered saline (PBS, pH; 7.4) and fixed in 4% paraformal-