available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

# Monoaminergic neuronal activity up-regulates BDNF synthesis in cultured neonatal rat astrocytes

*Damijana Mojca Jurič*, Špela Miklič, Marija Čarman-Kržan*

*Department of Pharmacology and Experimental Toxicology, Faculty of Medicine, University of Ljubljana, Korytkova 2, SI-1000 Ljubljana, Slovenia*

ARTICLE INFO

*Article history:*
Accepted 4 June 2006
Available online 7 July 2006

*Keywords:*
Monoamine
BDNF
Astrocyte
Rat

ABSTRACT

Astrocytes as an active part of the tripartite synapse can respond to the synaptically released neurotransmitters. Because brain-derived neurotrophic factor (BDNF) is produced by astrocytes, in addition to neurons, we focused our present study on the regulatory effects of monoamines noradrenaline (NA), serotonin (5-HT), and dopamine (DA) on the synthesis of BDNF protein in rat neonatal astrocytes from specific brain regions (cortex, cerebellum). All tested neurotransmitters are able to potently and transiently increase BDNF cellular contents; their maximal effects are dose and time dependent and differ between the two brain regions. In cultured cortical astrocytes, NA (1 μM; 6 h) elevates BDNF levels by a 4-fold, 5-HT (1 μM; 4 h) by a 2.3-fold, and DA (150 μM; 4 h) by a 2.2-fold. In cerebellar astrocytes, NA (1 μM; 4 h) increases BDNF content by a 4.7-fold, 5-HT (1 μM; 4 h) by a 1.7-fold, and DA (150 μM; 4 h) by a 1.4-fold. The initial increase in the BDNF levels return to basal levels when incubation with monoamines is extended beyond 12 h (for 5-HT) or 24 h (for NA and DA). Our results confirm the involvement of monoaminergic systems in the regulation of BDNF production in astrocytes and suggest the existence of a positive reciprocal interaction between monoaminergic neuronal activity and astrocytic neurotrophic support in neuron–astrocyte crosstalk, which has a dynamic role in mediating neuronal plasticity and trophic functions in the brain.



## 1. Introduction

Bidirectional communication between astrocytes and neurons is crucial for controlling activity-dependent and -independent structural and functional synaptic changes in the developing and adult brain (Araque et al., 1999; Fellin and Carmignoto, 2004, Elmariah et al., 2005), and neurotrophins play a distinctive role in these processes. Studies of astroglial responses to neurotransmitters have helped to elucidate the active role of astrocytes as a part of the tripartite synapse (Araque et al., 1999) in the regulation of neuronal activity, synaptic transmission, and neurotrophic support, as well as the possible involvement of these cells in the pathogenesis of neurodegenerative and mood disorders.

Several neurotrophic factors (i.e., neurotrophins), which can influence the above interactions, are synthesized in astrocytes, in addition to neurons (Elmariah et al., 2005). Astrocytes therefore represent an important local cellular source of trophic support during development and in the adult brain (Riley et al., 2004). They also express several neurotransmitter receptors that have the potential to be activated by synaptically released neurotransmitters (Kimelberg, 1995;

* *Corresponding author*. Fax: +386 1 543 73 31.
E-mail address: damijana-mojca.juric@mf.uni-lj.si (D. Mojca Jurič).

0006-8993/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2006.06.008

Porter and McCarthy, 1997). Activation of these receptors evokes a broad spectrum of responses in astroglial cells including calcium elevations (Verhratski et al., 1998; Fellin and Carmignoto, 2004) and the release of various gliotransmitters and other neuroactive substances including neurotrophic factors that enable astroglial cells to directly contribute to neuron–astrocyte network interactions through feedback or feedforward signaling to neurons (Araque et al., 1999, 2001; Fellin and Carmignoto, 2004).

Brain-derived neurotrophic factor (BDNF) is the most widely distributed neurotrophic factor in the CNS and has potent neurotrophic effects on a wide range of neuronal populations (noradrenergic, serotonergic, dopaminergic, cholinergic, and GABAergic neurons) (Friedman et al., 1993; Siuciak et al., 1994; Sklair-Tavron and Nestler, 1995; Mamounas et al., 1995). BDNF is predominantly produced by neurons, where the normal physiological levels are rapidly and potently regulated by ongoing neuronal activity (Zafra et al., 1992; da Penha Berzaghi et al., 1993, Lindholm et al., 1994; Thoenen, 1995). BDNF is also synthesized by astrocytes, which we demonstrated for neonatal rat cortical and cerebellar astrocytes in primary culture (Miklič et al., 2004). A growing body of evidence suggests that the synthesis of BDNF not only in neurons but also in astrocytes is stimulated by the released neurotransmitters. Increased noradrenaline (NA), serotonin (5-HT), and dopamine (DA) neurotransmission can induce neuronal BDNF expression within the cerebral cortex and the hippocampus (Fawcett et al., 1998; Ivy et al., 2003), thus identifying BDNF and monoamines as prominent signals in the brain that often cooperatively regulate neuronal survival, neuroplasticity, neurogenesis, and each other (for review, see Marien et al., 2004; Mattson et al., 2004). Monoaminergic regulation of BDNF synthesis in astrocytes is still poorly understood. In our previous study, we demonstrated that exposure of rat cortical astrocytes to DA (150μM) increased cellular BDNF content by a factor of 2, indicating that DA is a potent up-regulator of BDNF synthesis in astrocytes (Miklič et al., 2004). DA also potently stimulates secretion of BDNF protein from mice astrocytes (Inoue et al., 1997). Incubation of cultured rodent astrocytes with NA or β-adrenergic agonists markedly increases BDNF mRNA expression (Zafra et al., 1992; Schwartz and Nishiyama, 1994; Schwartz et al., 1994; Inoue et al., 1997), whereas the stimulatory effect of 5-HT on BDNF mRNA expression was only observed in C6 glioma cells (Meller et al., 2002). Regulation of astrocytic BDNF synthesis may differ between different brain regions because astrocytes express regionally specific spectra of neuroligand receptors (Shao et al., 1994), which contribute to the regional differences in response to neurotransmitters (Hansson et al., 1986).

We focused our present study on the regulatory effects of monoamines NA, 5-HT, and DA on the synthesis of BDNF protein in rat neonatal astrocytes from specific brain regions by examining dose dependency and short-term kinetics of their effects on cellular BDNF levels. Our results confirm the involvement of the monoaminergic systems in astrocytic BDNF synthesis and suggest the existence of a positive, reciprocal interaction between monoaminergic neuronal activity and astrocytic neurotrophic support in neuron–astrocyte crosstalk.

## 2. Results

In our present study, we examined the effects of monoamine neurotransmitters (NA, 5-HT, and DA) on BDNF synthesis in neonatal rat cortical and cerebellar astrocytes in primary culture. All neurotransmitters show a significant stimulatory influence on cellular levels of BDNF with differences in the dose dependency and short-term kinetics of their action. The results revealed that the examined monoamines have an important stimulatory role in the synthesis of BDNF in astrocytes.

### 2.1. *Effect of NA on BDNF cellular content in astrocytes*

According to the results of our previous study (Miklič et al., 2004), we first examined the dose dependency of the effects of monoamines after 4h of incubation in the cultured cells. Among the monoaminergic neurotransmitters tested, NA was the most effective elevator of cell levels of BDNF. Exposure of cultured cortical astrocytes for 4h to increasing concentrations of NA (1nM to 200μM) caused a marked dose-dependent increase in BDNF protein. The lowest concentration of NA (1nM) already significantly elevated BDNF levels by a factor of 1.3 (20.1 ± 7.0pg BDNF/mg protein) and the maximal effect was observed at 150μM of NA, where cultures contained 4.4-fold higher cellular levels of BDNF (70.1 ± 6.6pg BDNF/mg cell protein) than control cells (15.9 ± 0.3pg BDNF/mg protein) (Fig. 1). Because the concentration of NA causing maximal effect was relatively high (150μM), we repeated NA treatment of cortical astrocytes using a prolonged 6h incubation. The maximal effect

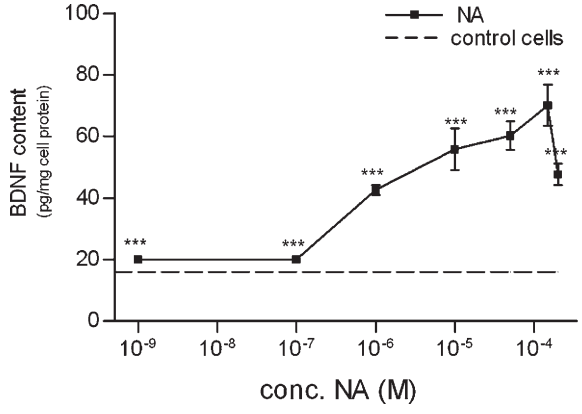


**Fig. 1 – Dose-dependent effect of NA on BDNF content in neonatal rat cortical astrocytes in primary culture. Cells were incubated for 4h in serum-free culture medium containing vehicle ($H_2O$) control cells or NA (1nM to 200μM). BDNF cellular was determined by the specific immunoassay as described in Experimental procedures. The values are the means ± SEM from 3 to 5 independent experiments with $n = 3–5$. Basal cellular content of BDNF in cortical astrocytes was 15.9 ± 0.3pg BDNF/mg protein (dashed line). Data were subjected to the Mann–Whitney test; the statistical significance of the difference relative to the corresponding control is indicated as $^{***}P < 0.001$.**