Neuropeptides 43 (2009) 275–282

Contents lists available at ScienceDirect

Neuropeptides

journal homepage: www.elsevier.com/locate/npep

Brain derived neurotrophic factor and neurotrophin-4 employ different intracellular pathways to modulate norepinephrine uptake and release in rat hypothalamus

M. Rodríguez Fermepin *, M. Trinchero, J. Minetto, A. Beltrán, B.E. Fernández

Cátedra de Fisiopatología, Facultad de Farmacia y Bioquímica, INFIBIOC, Universidad de Buenos Aires, CONICET, C1113AAD Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 25 November 2008
Accepted 3 June 2009
Available online 2 July 2009

Keywords:
Neurotrophins
Neurotransmission
PI3K
PLC
Hypothalamus
Trk receptors
NE uptake
NE release

ABSTRACT

Classical actions of the neurotrophin family are related to cellular survival and differentiation. Moreover, acute effects of neurotrophins have been reported. Although neurotrophins effects on synaptic transmission at central nervous system level have been largely studied, acute effects of neurotrophins on hypothalamic noradrenergic transmission are still poorly understood. Thus, we have studied the effects of the neurotrophin family members nerve growth factor (NGF), brain derived neurotrophic factor (BDNF) and neurotrophin-4 (NT-4) on norepinephrine (NE) neuronal uptake and its evoked release, as well as the receptor and the intracellular pathways involved in these processes in rat hypothalamus.

Present results indicate that BDNF increased NE uptake and decreased its evoked release through a mechanism that involve Trk B receptor and phospholipase C. Moreover, NT-4, also through the Trk B receptor, decreased NE uptake and its evoked release by activating phosphatidylinositol 3-OH-kinase. These effects were observed in whole hypothalamus as well as in the anterior hypothalamic zone. On the other hand, NGF did not modify noradrenergic transmission.

In conclusion, we showed for the first time that BDNF and NT-4 activate two different intracellular signalling pathways through a Trk B receptor dependent mechanism. Furthermore, present findings support the hypothesis that BDNF and NT-4 acutely applied, could be considered as modulators of noradrenergic transmission and thus may regulate hypothalamic physiological as well as pathophysiological responses.

1. Introduction

Noradrenergic neurotransmission is a complex process which includes norepinephrine (NE) biosynthesis, storage, release, interaction with its specific postsynaptic and presynaptic receptors, uptake and catabolism (Eisenhofer, 2001; Burgoyne and Morgan, 2003). Furthermore, NE uptake and release are the main processes that acutely regulate NE availability at the synaptic cleft and thus can modulate postsynaptic NE effects (Barclay et al., 2005; Bönisch and Brüss, 2006; Kubista and Boehm, 2006; Mandela and Ordway, 2006).

We have previously reported that different vasoactive peptides such as angiotensin II, angiotensin 1–7 and natriuretic peptides (Vatta et al., 1992, 1996; Gironacci et al., 2000; Rodríguez Fermepin et al., 2000) acutely applied regulate NE neuronal uptake and its release in the hypothalamus. Moreover, these peptides can be considered as neuromodulators of noradrenergic transmission. On the other hand, besides their acute effects, this group of vasoactive peptides is strongly related to cardiac hypertrophy, where they exert long term effects on cellular growth and proliferation (Barry et al., 2008; Katovich et al., 2008; Kumar et al., 2008). These antecedents allow us to hypothesise that other peptides which regulate cellular growth and proliferation, like neurotrophins (Lu et al., 2005), could also exert acute actions on noradrenergic transmission.

The neurotrophin family, which belongs to the neurotrophic factor superfamily, includes nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin-4 (NT-4) and neurotrophin-3. The members of this family exert their actions by binding to two types of cellular membrane receptors named tropomyosin-related kinase receptor family (Trk) and p75 neurotrophic receptor ($p75^{NTR}$) (Chao et al., 2006). Likewise, three members of Trk receptors have been described (Trk A, Trk B and Trk C), showing all of them different affinities for each neurotrophin. For instance Trk A presents higher affinity to NGF, Trk B to BDNF and NT-4, and Trk C to NT-3. On the other hand, every neurotrophin binds to $p75^{NTR}$ with similar affinity (Hempstead, 2002; Reichardt, 2006).

The interaction between neurotrophins and Trk receptors leads to the activation of different intracellular signalling pathways (Huang and Reichardt, 2003). The specificity of downstream Trk

* Corresponding author. Address: Junín 954 5° piso, C1113AAD Buenos Aires, Argentina. Address: Tel./fax: +54 11 4964 8268x33.
E-mail address: martinrf@ffyb.uba.ar (M. Rodríguez Fermepin).

0143-4179/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.npep.2009.06.001

receptor-mediated signalling depends on the expression and membrane trafficking of intermediates that finally regulate the activity of phospholipase C (PLC), phosphatidylinositol-3-OH-kinase (PI3K), mitogen-activated protein kinases (MAPKs) and Rho family GTPases (Reichardt, 2006).

The classical actions of neurotrophins are related to survival, growth, differentiation and proliferation of neuronal and glial cells (Lu et al., 2005). Moreover, after the early reports of Poo's group (Lohof et al., 1993; Stoop and Poo, 1995, 1996) many articles have emerged describing acute effects of neurotrophins on membrane excitability, activity-dependent synaptic plasticity and synaptic transmission (for review see Schuman (1999); Poo (2001); Lu (2004); Blum and Konnerth (2005)). Most of these studies were carried out in neuromuscular synapses, hippocampal neurons and visual cortex. Although neurotrophins expression was demonstrated in the hypothalamus (Nishio et al., 1994; Kawamoto et al., 1996; Yan et al., 1997), there are scarce reports describing acute effects of neurotrophins at this level (Tapia-Arancibia et al., 2004; Blum and Konnerth, 2005).

The hypothalamus is a brain area which contains integrative neural centres and receives abundant sympathetic input innervation (Kumar et al., 2007). Moreover, neuropeptides modulate the synaptic transmission in the hypothalamus and exert a critical role in the regulation of diverse output pathways closely related to the control of several physiological functions such as blood pressure, water and salt balance, thirst, body temperature and hormone secretion, as well as the modulation of pathophysiological responses to stress.

Thus, considering that neurotrophins family members could acutely modulate a sympathetic network involved in the control of physiological processes regulated by the hypothalamus, the aim of the present study was to analyze BDNF, NT-4 and NGF effects on NE neuronal uptake and release and to elucidate the intracellular signalling pathways involved in the modulation of noradrenergic neurotransmission.

2. Experimental procedures

Sprague–Dawley young adult male rats (2–3 month old) from the Department of Pathophysiology, Faculty of Pharmacy and Biochemistry, Buenos Aires, Argentina were used for the experiments. Female rats were discarded in order to avoid sexual cycle influence on NE metabolism (Renner et al., 1984). The animals were housed in steel cages, maintained in a controlled room at 22–24 °C with 12 h light/dark cycles (light from 7:00 am to 7:00 pm) and free access to tap water and food. Experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

2.1. Norepinephrine neuronal uptake

In order to determine the effects of BDNF, NT-4 and NGF on NE uptake in the hypothalamus, the experimental protocols were carried out according to techniques previously developed in our laboratory (Rodríguez Fermepin et al., 2000). Briefly, the animals were killed by decapitation between 10:00 and 11:30 am to avoid circadian changes. Each hypothalamus was quickly removed, dissected according to Palkovits and Brownstein (1988), cooled, minced and then transferred into a glass tube with a mesh of nylon fitted at the bottom to allow free interchange with the medium.

Several evidences indicated that anterior hypothalamic zone (AHZ) exerts opposite physiological effects than those of posterior hypothalamic zone (PHZ). In this way, the AHZ exerts a sympathoinhibitory regulation on central cardiovascular activity, while the PHZ induces sympathoexcitatory effects (Oparil et al., 1995). Then, in other set of experiments, hypothalami were divided in AHZ which included the paraventricular, periventricular and anterior hypothalamic nuclei, a segment of the ventromedial nucleus, the preoptic area and a portion of the median eminence, and in PHZ, which included the arcuate, supramammillary, mammillary and posterior hypothalamic nuclei, the dorsomedial and ventromedial nuclei, a segment of the lateral hypothalamic nucleus and the median eminence (Paxinos and Watson, 1986).

The tissues were pre-incubated at 37 °C for 30 min in a modified Krebs solution (MKS, pH 7.4) containing (mM) NaCl 118, KCl 4.7, $MgCl_2$ 1.2, NaH_2PO_4 1.0, $CaCl_2$ 2.5, EDTA-Na 0.004, dextrose 11.1, $NaHCO_3$ 25.0 and ascorbic acid 0.11, continuously bubbled with 95% O_2/5% CO_2. All reagents used were of analytical grade and obtained from standard sources.

To avoid ^{3}H-NE degradation and ^{3}H-NE non neuronal uptake, 100 μM of pargyline and 100 μM hydrocortisone (Sigma Chemical Co., St. Louis, USA) were respectively added in the incubation medium.

In order to determine which neurotrophin concentration was able to modified NE neuronal uptake, the tissues were incubated for 5 min in MKS containing 125 pM L-7-^{3}H-NE (New England Nuclear, Boston, MA, USA) in absence (control group) or presence (experimental groups) of increasing concentrations of BDNF, NT-4 or NGF (0.5; 5; 50 and 100 ng/ml) (Alomone Labs, Jerusalem, Israel). This concentration-range was based on the literature according to effective neurotrophin concentrations which show stimulating or inhibiting effects in the central nervous system, in *in vitro* experiments (Helke and Verdier-Pinard, 2000; Li and Keifer, 2008; Vaz et al., 2008).

Afterwards, to stop NE uptake and to avoid the possibility that remainder amounts of ^{3}H-NE could be uptaken by norepinephrine transporter (NET), the tissues were washed immediately four times for 15 min at 4 °C in cold MKS containing 15 μM desipramine (Sigma Chemical Co., St. Louis, MO, USA) to inhibit NET activity, and 4–6 μM nonradioactive NE (generously supplied by Dr. Lancelotti, Richet Laboratory, Buenos Aires, Argentina). After the washing period, tissues were homogenized and two aliquots were kept, one of them to determine tritium activity by scintillation counting method (Wallac 1214 RackBeta), and the other to measure protein content by Lowry's technique (Lowry et al., 1951). Results were expressed as dpm/μg prot ± SEM.

Once the concentration able to modify NE neuronal uptake for each neurotrophin was determined from the concentration–response curve, the receptor and intracellular pathways involved in these effects were studied. A similar protocol to that previously mentioned was used, except for it included an additional 15 min-inhibition period between the pre-incubation and incubation periods. The inhibitors and the concentrations used in these experiments were chosen according to previous reports of the literature (Li and Keifer, 2008; Busche et al., 2001; Secondo et al., 2003). Thus, to block Trk receptors and to inhibit PLC and PI3K activity, 200 nM K252a, 10 μM U 73122 and 25 μM LY 294002 (Alomone Labs, Jerusalem, Israel) dissolved in DMSO were respectively added to the incubation medium in the course of all inhibition and incubation periods. DMSO vehicle did not affect NE uptake or release.

2.2. Evoked norepinephrine neuronal release

Evoked ^{3} H-NE release was assessed according to slight modifications of the technique described by Vatta (Vatta et al., 1996). Briefly, minced rat hypothalami were pre-incubated at 37 °C for 30 min in MKS in the presence of 100 μM pargyline (to inhibit monoamine oxidase activity) and 100 μM hydrocortisone (to inhibit non neuronal NE uptake), and then NE stores were labelled with 125 pM L-7-^{3}H-NE for 30 min. After 6 consecutive 15-min washing periods with MKS, minced tissues were incubated for 5 min in MKS