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Short-term regulation of tyrosine hydroxylase activity and expression by endothelin-1 and endothelin-3 in the rat posterior hypothalamus

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Abstract

Brain catecholamines are involved in several biological functions regulated by the hypothalamus. We have previously reported that endothelin-1 and -3 (ET-1 and ET-3) modulate norepinephrine release in the anterior and posterior hypothalamus. As tyrosine hydroxylase (TH) is the rate-limiting enzyme in catecholamine biosynthesis, the aim of the present work was to investigate the effects of ET-1 and ET-3 on TH activity, total enzyme level and the phosphorylated forms of TH in the rat posterior hypothalamus. Results showed that ET-1 and ET-3 diminished TH activity but the response was abolished by both selective ET_A and ET_B antagonists (BQ-610 and BQ-788, respectively). In addition ET_A and ET_B selective agonists (sarafotoxin S6b and IRL-1620, respectively) failed to affect TH activity. In order to investigate the intracellular signaling coupled to endothelins (ETs) response, nitric oxide (NO), phosphoinositide, cAMP/PKA and CaMK-II pathways were studied. Results showed that N^{ω}-nitro-L-arginine methyl ester and 7-nitroindazole (NO synthase and neuronal NO synthase inhibitors, respectively), 1H-[1,2,4]-oxadiazolo[4,3-alpha]quinozalin-1-one and KT-5823 (soluble guanylyl cyclase, and PKG inhibitors, respectively) inhibited ETs effect on TH activity. Further, sodium nitroprusside and 8-bromoguanosine-3′,5′-cyclic monophosphate (NO donor and cGMP analog, respectively) mimicked ETs response. ETs-induced reduction of TH activity was not affected by a PKA inhibitor but it was abolished by PLC, PKC and CaMK-II inhibitors as well as by an IP_3 receptor antagonist. On the other hand, both ETs did not modify TH total level but reduced the phosphorylation of serine residues of the enzyme at positions 19, 31 and 40. Present results suggest that ET-1 and ET-3 diminished TH activity through an atypical ET or ET_C receptor coupled to the NO/cGMP/PKG, phosphoinositide and CaMK-II pathways. Furthermore, TH diminished activity may result from the reduction of the phosphorylated sites of the enzyme without changes in its total level. Taken jointly present and previous results support that ET-1 and ET-3 may play a relevant role in the modulation of catecholaminergic neurotransmission in the posterior hypothalamus of the rat.

Keywords: ET_A receptor; ET_B receptor; ET_C receptor; Atypical ET receptor; Nitric oxide pathway; Phosphoinositide pathway; CaMK-II; Catecholaminergic transmission

1. Introduction

Endothelins (ETs) are 21 amino acid peptides that exist in three isoforms, ET-1, ET-2 and ET-3, and display vasoactive as well as growth regulatory properties [1,2]. The isoforms, encoded by different genes, derive from a larger precursor molecule named pre-pro-ET that is first cleaved to generate big-ETs and then hydrolyzed by an endothelin-converting enzyme to produce the biological active peptides [1–3]. Two well characterized G-protein-coupled receptors (GPCRs) mediate most of ETs biological actions, ET_A that exhibits higher affinity for ET-1 than for ET-2 and ET-3, and ET_B, that displays similar affinity for the three isoforms [2,4–6]. Several authors based on physiological and pharmacological studies support the existence of other ETs

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receptors termed atypical receptors (ET_{Ax} and ET_{Bx}) based on atypical responses observed in the presence of selective agonists and antagonists of the conventional ET_A and ET_B receptors [7,8]. In addition a third receptor subtype named ET_C, which displays high affinity for ET-3 was reported in *Xenopus laevis*, and although several studies in mammals support its existence, it has not been cloned in this specie yet [9–11]. The activation of ETs receptors triggers a wide and complex variety of intracellular signaling pathways [2,6]. ETs system components are expressed in several neuronal structures throughout the CNS including the hypothalamus, which is an integrative and regulatory center of diverse biological functions including the cardiovascular regulation [2,6,12,13].

The posterior hypothalamic region (PHR) is recognized as a sympatho-excitatory area intimately related to the central regulation of the cardiovascular function [12,13]. Thus, the stimulation of the posterior hypothalamic area increases sympathetic outflow and blood pressure, whereas it attenuates barorreflex-induced bradycardia [12]. Impairment of norepinephrine metabolism in the posterior hypothalamus has been associated with the development and/or maintenance of hypertension [12].

Tyrosine hydroxylase (TH) is the rate-limiting enzyme in the biosynthesis of catecholamines [14–16]. The enzyme is highly regulated by both, short and long-term mechanisms. The short-term regulation involves the classical feedback by end products, the allosteric regulation, and a postranslational mechanism mediated by reversible phosphorylation of serine (Ser) residues in the N-terminal regulatory domain [14,16,17]. The long-term regulation includes, translational and transcriptional mechanisms such as, RNA alternative processing and stability, as well as gene expression control [16,17]. It has been well established that substances that modify the catecholaminergic function at different levels induce changes in TH mRNA and/or the enzyme activity [16,17].

Several reports show that ETs regulate a wide variety of biological functions besides cardiovascular activity [2,5,18]. Increasing evidence supports that these peptides may function as neurotransmitters and/or regulatory neuropeptides within the CNS. ET-1 increases dopamine release in the rat striatum and ET-3 evokes catecholamine release in cortical and striatal brain slices in the rat [19,20]. Furthermore, we reported that both ETs modulate norepinephrine (NE) release in the anterior and posterior hypothalamus of the rat [9,21]. Recently, we showed that both ET-1 and ET-3 decrease TH activity in the anterior hypothalamus through the activation of ET_B receptors coupled to the nitric oxide (NO)/cGMP/PKG pathway [22].

In the present study we sought to establish the role of ET-1 and ET-3 on the short-term regulation of TH activity in the PHR and to determine the receptors and intracellular mechanisms involved. In addition, the expression of total TH and phosphorylated forms of the enzyme were also determined. Our findings showed that ET-1 and ET-3 reduced TH activity in the PHR not through the conventional ET_A and ET_B receptors but through an atypical ET_{Ax}/ET_{Bx} or ET_C receptor coupled to the NO, phosphoinositide and CaMK-II pathways. Furthermore, both ETs diminished the phosphorylation of TH at Ser-19, Ser-31 and Ser-40 sites without affecting total protein levels. Present findings support that ETs play a relevant role in the modulation of the catecholaminergic transmission in the PHR.

2. Materials and methods

2.1. Animals and chemicals

Male Sprague–Dawley rats weighing between 250 and 300 g (from the Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires) were used in the experiments. The rats were housed in steel cages and maintained at a temperature between 20 and 23 °C in a controlled room with a 12 h light–dark cycle. All animals were fed *ad libitum* and had free access to water.

The following drugs and reagents were used: L-[3,5–^{3}H] tyrosine (1.70 T Bq/mmol of specific activity) and PVDF membrane (GE Healthcare, Amersham Biosciences, UK); ET-1, ET-3, BQ-610, and BQ-788 (American Peptides, CA, USA); catalase, GF-109203x, KN-62, L-DOPA, H-89, L-tyrosine, suramin (SMN), 6-methyl-tetrahydrobiopterin, minimum essential media (MEM) amino acid solution, and basal medium Eagle vitamin solution (MP Biomedicals, CA, USA); anti-actin polyclonal antibody (Ab) (Actin-Ab), 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP-NBT), 8-bromoguanosine-3′,5′-cyclic monophosphate (8-Br-cGMP), N^{ω}-nitro-L-arginine methyl ester (L-NAME), 1H-[1,2,4] oxadiazolo[4,3-alpha]quinoxalin-1-one (ODQ), protease inhibitor cocktail, sodium nitroprusside dehydrate (SNP), streptavidin–alkaline phosphatase conjugate and U-73122 (Sigma, MO, USA); 2-APB, IRL-1620, 7-nitroindazole (7-NI), and sarafotoxin S6b (SRTx-b) (Calbiochem, CA, USA). Mouse anti-TH monoclonal Ab (TH-Ab), and rabbit anti-TH phospho-Ser-19, -31 and -40 (19 Ser-P, 31 Ser-P and 40 Ser-P, respectively), biotinylated anti-mouse Ab, and anti-rabbit Ab (Chemicon, USA). Other reagents were of analytical or molecular biology quality and obtained from standard sources.

2.2. Experimental design

Experiments were performed following the recommendations of the Guide for the Care and Use of Laboratory Animal (NIH Publication No. 85–23 1985, Revised 1996). Animals were decapitated between 9:00 and 12:00 h, hypothalami removed, and PHR dissected under a magnifier glass. Tissues were pre-incubated in a Dubnoff incubator for 30 min at 37 °C in Krebs bicarbonate solution supplemented with MEM amino acid solution and basal medium Eagle vitamin solution (KBSS), pH 7.4, and bubbled with a gas mixture (95% O_2 and 5% CO_2) under continuous shaking. To determine the effects of ET-1 or ET-3 (10 nM) on TH activity, tissues were incubated for 30 min in the presence or in the absence of the ETs (experimental and control groups, respectively). The following drugs were added 15 min before the incubation period: 100 nM BQ-610 (ET_A receptor antagonist), 100 nM BQ-788 (ET_B receptor antagonist), 500 nM SMN (G-protein inhibitor), 10 μM L-NAME (NO synthase (NOS) inhibitor), 10 μM 7-NI (neuronal NOS