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Involvement of nitric oxide pathways in short term modulation of tyrosine hydroxylase activity by endothelins 1 and 3 in the rat anterior hypothalamus

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Abstract

The ability of endothelins 1 and 3 (ET-1 and ET-3) to reduce neuronal norepinephrine release through ETB receptor activation involving nitric oxide (NO) pathways in the rat anterior hypothalamus region (AHR) was previously reported. In the present work, we studied the effects of ET-1 and -3 on tyrosine hydroxylase (TH) activity and the possible involvement of NO pathways. Results showed that ET-1 and -3 (10 nM) diminished TH activity in AHR and this effect was blocked by a selective ETB receptor antagonist (100 nM BQ-788), but not by a ET_A receptor antagonist (BQ-610). To confirm these results, 1 μM IRL-1620 (ET_B agonist) reduced TH activity whereas 300 nM sarafotoxin S6b falled to modify it. N^{ω}-Nitro-L-arginine methyl ester (10 μM), 7-nitroindazole (10 μM), 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-ona (10 μM), KT5823 (2 μM), inhibitors of nitric oxide synthase, neuronal nitric oxide synthase, NO-sensitive-guanylyl cyclase, and protein kinase G, respectively, did not modify the reduction of TH activity produced by ETs. In addition, both 100 μM sodium nitroprusside and 50 μM 8-bromoguanosine-3′,5′-cyclic monophosphate (NO donor and guanosine-3′,5′-cyclic monophosphate analog, respectively) diminished TH activity. Present results showed that ET-1 and ET-3 diminished TH activity through the activation of ET_B receptors involving the NO/guanosine-3′,5′-cyclic monophosphate/protein kinase G pathway. Taken jointly present and previous results it can be concluded that both ETs play an important role as modulators of norepinephrine neurotransmission in the rat AHR.

Keywords: Tyrosine hydroxylase; Endothelin-1; Endothelin-3; Endothelin-A receptor; Endothelin-B receptor; Nitric oxide pathways; Anterior hypothalamus

Endothelins (ETs) are peptides of 21 amino acids, that exist in three isoforms, ET-1, ET-2 and ET-3, and display vasoactive and growth regulatory properties [1,2]. The three peptides derive from a larger precursor molecule termed preproET that is first cleaved to generate bigETs (1, 2, and 3). These inactive intermediates are then hydrolyzed by an endothelin-converting enzyme to produce the biological active peptides [1–3]. The biological actions of ETs are mediated by at least two well characterized G-protein-coupled receptors, ET_A receptor, that shows higher affinity for ET-1 than for ET-2 and ET-3, and ET_B receptor, that displays similar affinity for the three ETs [2,4]. Activation of ET receptors triggers an extense and complex variety of intracellular signaling pathways including phospholipase C/inositol triphosphate/diacylglycerol, Ca^{2+}, adenylyl cyclase/adenosine-3′,5′-cyclic monophosphate (cAMP), nitric

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oxide (NO), and guanylyl cyclase/guanosine-3′,5′-cyclic monophosphate (cGMP) [2,4]. In turn, messengers activate their own biochemical pathways, compromising different protein kinases, as Ca^{2+} calmodulin-dependent protein kinase II (CaMK-II), cAMP-dependent protein kinase (PKA), cGMP-dependent protein kinase (PKG), Ca^{2+}/phospholipid-dependent protein kinase (PKC), and mitogen-activated protein kinases (MAPK) [2,4].

Despite the well-known vascular localization, ET-like inmunoreactivity, ET mRNA and ET binding sites are also found in the central nervous system (CNS), especially in the hypothalamus which is an integrative and regulatory center of the cardiovascular function [2–4]. Elevated expression of ET-1 and ET-3 as well as high densities of ET-binding sites were shown in the hypothalamus [2,4,5]. In addition, ET receptors coexist with catecholaminergic neurons in hypothalamus [6]. The hypothalamus has two important antagonistic regions related to blood pressure regulation, the anterior hypothalamic region (AHR), where the enhancement of catecholaminergic activity induces peripheral vasodilatation, leading to a decrease in blood pressure [7–9], and the posterior hypothalamic region (PHR), where an increase in catecholaminergic activity evokes peripheral vasoconstriction, resulting thereby in blood pressure rise [9,10].

Tyrosine hydroxylase (TH), a specific marker for catecholaminergic neurons, catalyzes the hydroxylation of L-tyrosine to L-DOPA, being the rate-limiting step in the production of catecholamines [11,12]. TH activity is tightly regulated at different levels: control of gene transcription, RNA alternative processing, mRNA stability, and direct modulation by catecholamine feedback inhibition and protein-serine-kinase covalent modification [12]. The last regulatory mechanism of TH activity is mediated by protein-serine-kinases like PKA, CaMK-II, PKC, PKG, and MAPK [11–13], enzymes that could be activated by ETs.

Several studies report the existence of direct interactions between ETs and brain neurons supporting that these peptides play a role as neurotransmitters or neuromodulators within the CNS. Thus, ET-1 induces dopamine release mediated by the ET_B receptor in the rat striatum [14]. Furthermore, ET-3 evokes the release of catecholamines from cortical and striatal brain slices in the rat [15]. Recently, we reported that both, ET-1 and ET-3, modulates neuronal norepinephrine (NE) release in anterior hypothalamus of the rat through the activation of ET_B receptor involving NO pathway [16].

In the present study, we sought to establish the short term effect of ET-1 and ET-3 on TH activity in the AHR of the rat hypothalamus as well as the receptors involved. In addition, the possible involvement of NO pathways was also investigated. Our findings showed that ET-1 and ET-3 reduced TH activity in AHR through ET_B receptor activation and NO pathways. Taken jointly present and previous findings on neuronal NE release [16] support that ETs play an important role in the modulation of NE transmission in the AHR.

Materials and methods

Animals and chemicals. Male Sprague–Dawley rats weighing between 250 and 300 g (from the Faculty of Pharmacy and Biochemistry, University of Buenos Aires) were used. 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, according to local regulations (Servicio Nacional de Sanidad y Calidad Agroalimentaria of Argentina, Resolution 617/2002). All animals had free access to water and commercial chow.

The following drugs, reagents, and kits were used: L-[3,5-^{3}H]tyrosine (1.70 TBq/mmol of specific activity, Amersham Biosciences, England); ET-1, ET-3, BQ-610, and BQ-788 (Peninsula Lab., CA, USA); Catalase, L-DOPA, H-89, L-tyrosine, suramin (SMN), tetrahydrobiopterin, minimum essential media (MEM) amino acid solution, and basal medium Eagle vitamine solution (ICN Biomedicals, USA); 8-bromoguanosine-3′,5′-cyclic monophosphate (8-Br-cGMP), N^{ω}-nitro-L-arginine methyl ester (L-NAME), 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ), and sodium nitroprusside dihydrate (SNP) (Sigma, MO, USA); IRL-1620, 7-nitroindazole (7-NI), and sarafotoxin S6b (SRTx-b) (Calbiochem, CA, USA). Other reagents were of analytical or molecular biology quality and obtained from standard sources.

Experimental design. Experiments were performed in accordance with 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 AM, hypothalami were removed, and AHR was dissected according to Palkovits and Brownstein [17]. 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 vitamine solution (KBSS), pH 7.4, and bubbled with a gas mixture (95% O_2 and 5% CO_2) under continuous shaking. To determinate the acute effects of ET-1 or ET-3 (10 nM) on TH activity, AHR was incubated for 30 min in the presence or in the absence (experimental and control groups, respectively) of the ETs.

The following drugs were added 15 min before incubation with ETs: 100 nM BQ-610 (ET_A receptor antagonist), 100 nM BQ-788 (ET_B receptor antagonist), 500 nM SMN (G proteins inhibitor), 10 μM L-NAME (nitric oxide synthase (NOS) inhibitor), 10 μM 7-NI (neuronal NOS inhibitor), 10 μM ODQ (NO-sensitive- guanylyl cyclase inhibitor), and 2 μM KT5823 (PKG inhibitor). On the other hand, the following agonists were added during 30 min of incubation: 300 nM SRTx-b and 1 μM IRL-1620 (ET_A and ET_B receptor agonist, respectively), 100 μM SNP and 8Br-cGMP (NO donor and cGMP analog, respectively).

Determination of TH activity. TH activity was determined by the method of Reinhard et al. [18]. Briefly, after the incubation AHR were homogenized in 500 μl buffer (5 mM KH_2PO_4 and 0.2% Triton X-100, pH 7.0). After saving an aliquot for protein determination, samples were centrifuged for 10 min at 10,000*g* at 4 °C and an aliquot of supernatant incubated for 20 min at 37 °C with 50 mM Hepes (pH 7.0), in the presence of 15 nmol L-tyrosine containing 0.5 μCi ^{3}H[3,5]tyrosine, 420 mM β-mercaptoethanol, 1000 U catalase, and 0.75 mM 6-methyl-tetrahydrobiopterin. The reaction was stopped by the addition of 1 ml of 7.5% activated charcoal suspension in 1 N HCl. The final mixture was vortexed and centrifuged at 500*g* for 10 min. The supernatant was separated to determine the radioactivity of ^3H_2O by conventional scintillation methods. Blank values were obtained by omitting the 6-methyl-tetrahydrobiopterin. ^3H_2O recovered was determined as described by Reinhard et al. [18]. Results are expressed as the percentage of control group ± SEM.