Available online at www.sciencedirect.com
SCIENCE DIRECT
Brain Research 1059 (2005) 139 – 148
BRAIN RESEARCH
www.elsevier.com/locate/brainres

Research Report

Urotensin II acts as a modulator of mesopontine cholinergic neurons

Stewart D. Clark [a,f], Hans-Peter Nothacker [b], Charles D. Blaha [c], Christopher J. Tyler [d], Dee M. Duangdao [b], Stephen L. Grupke [d], David R. Helton [e], Christopher S. Leonard [d], Olivier Civelli [a,b,*]

[a]Department of Developmental and Cell Biology, University of California, Irvine, CA 92697-4625, USA
[b]Department of Pharmacology, University of California, 369 MSRII, Irvine, CA 92697-4625, USA
[c]Department of Psychology, University of Memphis, Memphis, TN 38152, USA
[d]Department of Physiology, New York Medical College, Valhalla, NY 10595, USA
[e]Cenomed Inc., 22865 Lake Forest Drive, Lake Forest, CA 92630, USA
[f]Laboratory of Molecular Neurobiology, Centre for Addiction and Mental Health, 250 College Street, Toronto, Ontario, Canada M5T 1R8

Accepted 9 August 2005
Available online 23 September 2005

Abstract

Urotensin II (UII) is a vasomodulatory peptide that was not predicted to elicit CNS activity. However, because we have recently shown that the urotensin II receptor (UII-R) is selectively expressed in rat mesopontine cholinergic (MPCh) neurons, we hypothesize that UII may have a central function. The present study demonstrates that the UII system is able to modulate MPCh neuron activity. Brain slice experiments demonstrate that UII excites MPCh neurons of the mouse laterodorsal tegmentum (LDTg) by activating a slow inward current. Furthermore, microinfusion of UII into the ventral tegmental area produces a sustained increase in dopamine efflux in the nucleus accumbens, as measured by in vivo chronoamperometry. In agreement with UII activation of MPCh neurons, intracerebroventricular injections of UII significantly modulate ambulatory movements in both rats and mice but do not significantly affect startle habituation or prepulse inhibition. The present study establishes that UII is a neuromodulator that may be exploited to target disorders involving MPCh dysfunction.

Theme: Neurotransmitters, modulators, transporters, and receptors
Topic: Peptides: anatomy and physiology

Keywords: Urotensin II; Mesopontine cholinergic; Electrophysiology; Chronoamperometry; Locomotion; Prepulse inhibition

1. Introduction

Urotensin II (UII) is a vasomodulatory peptide that has been recently shown to be the natural ligand of a G protein-coupled receptor formerly called GPR14 [1,25,28,30], now known as the urotensin II receptor (UII-R). Although UII is well known for its vascular effects (for review see [9]), several reports have indicated that it may also have effects in the central nervous system [7,11,17,25,27]. In particular, we have shown that the UII-R is selectively expressed in mesopontine cholinergic (MPCh) neurons of the rat brain [7], inferring that UII can modulate the activity of MPCh neurons. In addition, the fact that UII-R activation elicits increases in cytoplasmic calcium ion concentrations in vitro [1,24,30] indicates that UII could promote cell depolarization. Studies have measured the effects of UII after injections into the brain [17,23,25,27], and our recent data indicate UII promotes REM sleep by exciting MPCh neurons of the pedunculopontine tegmental nucleus (PPTg; [18]). Here, we

Abbreviations: Icv, intracerebroventricular; LDTg, laterodorsal tegmentum; MPCh, mesopontine cholinergic; NAc, nucleus accumbens; PPTg, pedunculopontine tegmentum; PPI, prepulse inhibition; UII, urotensin II; UII-R, urotensin II receptor; VTA, ventral tegmental area

* Corresponding author. Department of Pharmacology, University of California, 369 MSRII, Irvine, CA 92697-4625, USA. Fax: +1 949 824 4855.
E-mail address: ocivelli@uci.edu (O. Civelli).

0006-8993/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2005.08.026

have extended this analysis by examining UII actions on an additional group of MPCh neurons in the laterodorsal tegmental (LDTg) nucleus and on other functions associated with MPCh neurons.

Activation of the PPTg and LDTg is thought to contribute to a number of behavioral and physiological phenomena. For example, Garcia-Rill et al. [15] demonstrated that electrical stimulation of these regions elicited stepping behavior in the rat. In addition, lesions or electrical inhibition of the mesopontine tegmentum in non-human primates can induce ataxia [22,29]. Together, these studies have suggested that the brainstem area encompassing MPCh neurons profoundly influences motor function in mammals [38]. MPCh neurons have also been implicated in the modulation of the sensorimotor reactivity. Garcia-Rill et al. [16] predicted that activation of neurons in the mesopontine would reduce habituation of the startle reflex to a loud auditory stimulus. MPCh neurons are also intimately involved in prepulse inhibition (PPI), a phenomenon in which the amplitude of the startle reflex is reduced by the presence of a preceding muted warning stimulus (for review see [10,20]). Elucidating the role of MPCh neurons in PPI is of great interest because PPI deficits are a hallmark and diagnostic symptom in many neuropsychiatric disorders (for review see [20]). It is believed that the MPCh input from the PPTg to the caudal pontine nucleus inhibits the startle reflex [21], as destruction of the PPTg impairs PPI [21,34]. Therefore, we predict that agents such as UII, which potentially modulate MPCh activity, should influence both the startle reflex and PPI.

Several diverse techniques were employed to establish UII as a modulator of MPCh function. First, the response of MPCh neurons to UII stimulation was determined in vitro. Then, to test whether UII activates neurons in vivo, chronoamperometric recordings of dopamine efflux in the nucleus accumbens (NAc) were carried out after UII microinjection into the ventral tegmental area (VTA). Electrical stimulation of MPCh neurons located in the region of the PPTg and LDTg has been shown to induce increases in basal dopamine efflux in the striatum and NAc [12,13]. Furthermore, the MPCh neurons constitute the only known cholinergic excitatory input to the VTA dopaminergic cells [31]. Given that our previous study showed that the VTA contains binding sites for radiolabeled UII [7], it was expected that UII injected into the VTA would produce changes in NAc dopamine efflux. Lastly, behavioral paradigms associated with MPCh activity were used to extend the anatomical and neurochemical findings.

2. Materials and methods

2.1. In situ hybridization

Sources of the following materials were: bovine serum albumin, polyvinylpyrolidone, poly-L-lysine, RNase A (Sigma, St. Louis, MO); pBluescript SK (Stratagene, La Jolla, CA); pCR 4-TOPO (Invitrogen, Carlsbad, CA); anti-digoxygenin (dig)-AP Fab antibody, dig-dUTP, Genius system nonradioactive nucleic acid detection kit, restriction enzymes, T3, T7 polymerases, proteinase K, and yeast tRNA (Roche Molecular Biochemicals, Indianapolis, IN); formamide (Fluka, Ronkonkoma, NY); dextran sulfate (Pharmacia, Piscataway, NJ); sodium acetate (Fisher Scientific, Pittsburgh, PA); Hyperfilm βmax (Amersham, Arlington Heights, IL); nuclear track emulsion (NTB2) (Kodak, Rochester, NY); [^{35}S]-Uridine triphosphate ([^{35}S]-UTP) (Dupont NEN, Boston, MA).

2.1.1. Tissue preparation

CD-1 mice weighing approximately 18–30 g (Charles River, San Diego, CA), kept on a 12-h light/dark cycle with food and water ad libitum, were decapitated and brains were removed. Tissues were frozen immediately by immersion in −20 °C isopentane, then stored at −70 °C until used. All tissue removal procedures were approved by the Institutional Animal Care and Use Committee and were consistent with Federal guidelines. Twenty-micron sections were cut using a cryostat and mounted onto Vectabond (Vector Laboratories) coated slides. Fixation was performed (4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4) for 1 h at 22 °C. Slides were then rinsed in PB, air dried, and subsequently stored with desiccate at −20 °C.

2.1.2. Hybridization

All sections and probes were prepared as described by Clark et al. [7]. Briefly, sections were incubated with proteinase K (1 mg/mL) for 10 min at 22 °C, then acetylated, and dehydrated through graded ethanol, followed by air-drying. Sections were exposed to a 1:1 dilution of digoxigenin-labeled anti-sense choline acetyltransferase (ChAT) riboprobe (0.2 ng/mL):[^{35}S]-labeled UII-R sense or anti-sense probes (2×107 cpm/mL) in hybridization solution (50% formamide, 10% dextran sulfate, 0.02% Ficoll, 0.02% polyvinylpyrolidone, 0.02% BSA, 500 mg/mL tRNA, 10 mM DTT, 0.3 M NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA, pH 8.0) then incubated overnight at 60 °C. Incubation with RNase A (20 mg/mL) for 30 min at 37 °C followed the hybridization. The sections were then washed 2×5 min and 2×10 min in solutions of decreasing salinity at 22 °C and a 30 min wash in $0.1 \times$ SSC at 68 °C. After the hot wash, the slides were processed for digoxigenin labeling as per the manufacturer's instructions (Roche Molecular Biochemicals, Indianapolis, IN). Slides were then apposed to Bmax film for development of autoradiograms. Following film development, slides were coated with 3% parlodion in isoamylacetate and dipped in liquid NTB2 emulsion on the reference date of the [^{35}S]-UTP. Slides for each probe were incubated according to the exposure time on film: 1 day on film, 1 week incubation with emulsion. After the appropriate exposure period, slides were developed in Kodak D-19, fixed, coverslipped, and analyzed.