Behavioural Brain Research 278 (2015) 271–279

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Intra-ventral tegmental area microinjections of urotensin II modulate the effects of cocaine

L.E. Mueller[a], M.A. Kausch[a], T. Markovic[a], D.A.A. MacLaren[a], D.M. Dietz[a,c], J. Park[d], S.D. Clark[a,b,c,*]

[a] Department of Pharmacology and Toxicology, State University of New York at Buffalo, NY 14214, USA
[b] Department of Psychology, State University of New York at Buffalo, NY 14214, USA
[c] Research Institute on Addictions, State University of New York at Buffalo, NY 14214, USA
[d] Department of Biotechnology and Clinical Laboratory Sciences, State University of New York at Buffalo, NY 14214, USA

HIGHLIGHTS

- Urotensin II (UII) is a neuropeptide not previously linked to reward behaviors.
- High concentrations of UII in the VTA produces conditioned place preference (CPP).
- Low concentrations of UII potentiates sub-threshold doses of cocaine to produce CPP.
- UII potentiates cocaine-mediated release of dopamine in the nucleus accumbens.
- The endogenous UII-system may play a role in modulating reward systems.

ARTICLE INFO

Article history:
Received 28 July 2014
Received in revised form
13 September 2014
Accepted 19 September 2014
Available online 1 October 2014

Keywords:
Urotensin II
Pedunculopontine tegmentum
Laterodorsal tegmentum
Conditioned place preference
Cocaine

ABSTRACT

Although the peptide urotensin II (UII) has well studied direct actions on the cardiovascular system, the UII receptor (UIIR) is expressed by neurons of the hindbrain. Specifically, the UIIR is expressed by the cholinergic neurons of the laterodorsal tegmentum (LDTg) and the pedunculopontine tegmentum (PPTg). These neurons send axons to the ventral tegmental area (VTA), for which the PPTg and LDTg are the sole source of acetylcholine. Therefore, it was hypothesized that UIIR activation within the VTA would modulate reward-related behaviors, such as cocaine-induced drug seeking. Intra-VTA microinjections of UII at high concentrations (1 nmole) established conditioned place preference (CPP), but also blocked cocaine-mediated CPP (10 mg/kg). When rats received systemic sub-effectual doses of cocaine (7.5 mg/kg) with intra-VTA injections of 1 or 10 pmole of UII CPP was formed. Furthermore, the second endogenous ligand for the UIIR, urotensin II-related peptide, had the same effect at the 10 pmole dose. The effects of low doses of UII were blocked by pretreatment with the UIIR antagonist SB657510. Furthermore, it was found that intra-VTA UII (10 pmole) further increased cocaine-mediated (7.5 mg/kg) rises in electrically evoked dopamine in the nucleus accumbens.

Our study has found that activation of VTA-resident UIIR produces observable behavioral changes in rats, and that UIIR is able to modulate the effects of cocaine. In addition, it was found that UIIR activation within the VTA can potentiate cocaine-mediated neurochemical effects. Therefore, the coincident activation of the UII-system and cocaine administration may increase the liability for drug taking behavior.

Abbreviations: aCSF, artificial cerebral spinal fluid; CPP, conditioned place preference; DA, dopamine; GnRH, gonadotropin-releasing hormone; i.p., intraperitoneal injection; LDTg, laterodorsal tegmentum; NAc, nucleus accumbens; PPTg, pedunculopontine tegmentum; SN, substantia nigra; UII, urotensin II; UIIR, urotensin II receptor; URP, urotensin II-related peptide; VTA, ventral tegmental area.

* Corresponding author at: Department of Pharmacology and Toxicology, State University of New York at Buffalo, NY 14214, USA. Tel.: +1 7168293810; fax: +1 7168292800.
E-mail address: stewartc@buffalo.edu (S.D. Clark).

http://dx.doi.org/10.1016/j.bbr.2014.09.036
0166-4328/

1. Introduction

In the last decade the discovery of a number of neuropeptides has greatly increased our understanding of brain function. Moreover, a number of these novel neuropeptides have been implicated in addiction- and reward-related behaviors (e.g. melanin-concentrating hormone, neuropeptide S, hypocretin [7,25,35,52,53]). Another neuropeptide which holds promise in modulating reward-related behaviors is urotensin II (UII). The UII-system is comprised of one receptor (a G protein-coupled receptor (GPCR)) and two ligands (UII and UII-related peptide (URP)). In the mammalian brain the urotensin II receptor (UIIR) is selectively expressed in the mesopontine tegmentum by cholinergic neurons of the laterodorsal tegmentum (LDTg) and the pedunculopontine tegmentum (PPTg) [10]. These brain structures are widely interconnected with the basal ganglia [33], and their involvement in motivated and reward-related behavior [1–5,13,39–42,60,63] is thought to be primarily mediated through connections to midbrain DA systems.

The ventral tegmental area (VTA) is a key component of the so called "Reward Pathway" and it is believed that dysregulation of the VTA-accumbens-prefrontal cortex (PFC) circuitry is a critical neuronal mechanism of addiction. The LDTg is a major excitatory input to the VTA, with the caudal PPTg also innervating the VTA [20,38]. Moreover, the PPTg and LDTg are the sole cholinergic input to the VTA [31] and recently have been shown to directly innervate the nucleus accumbens (NAc; [12]). The inactivation of the LDTg or the PPTg reduces the phasic firing of VTA dopaminergic neurons [26,43], which is thought to be sufficient for behavioral conditioning [56]. In addition, previous to the LDTg studies, it was found that PPTg activation drives phasic firing of dopaminergic VTA neurons [16]. However, this effect was abolished when the LDTg was inactivated [26]. The present understanding is that input from the LDTg and PPTg is important for both the tonic to phasic firing of the VTA dopaminergic neurons and it is this phasic firing that is important in the learning of reward-related behaviors. As for the effects of cholinergic agents within the VTA, LDTg electrically evoked dopamine release in the NAc is blocked by intra-VTA cholinergic antagonists [17] and intra-VTA carbochol can produce conditioned place preference [21,63]. Moreover, cholinergic input has been implicated in the control of the firing pattern of dopaminergic VTA neurons [29]. Therefore, the evidence suggests that the direct modulation of the cholinergic terminals within the VTA would modulate reward-related behaviors.

Evidence for a role of the mesopontine tegmentum in reward-related behaviors comes from studies demonstrating that non-selective (ibotenic acid) lesioning of the PPTg blocks the acquisition of morphine conditioned place preference (CPP), but not cocaine CPP [3,39,41], reduces the breakpoint for heroin in the progressive ratio schedule [42], impairs the acquisition of amphetamine self-administration [1] and lesioning the posterior PPTg increases nicotine self-administration [2]. In addition, non-selective inactivation of the PPTg (e.g. by muscimol) blocks the ability to form new associations between actions and outcomes [27]. The accumulated evidence suggests that the LDTg/PPTg play an important role in reward-related behaviors.

The expression of the UIIR by cholinergic LDTg/PPTg neurons may allow for the specific pharmacological modulation of this brain region. We previously found that activation of UIIR in the VTA (expressed by presynaptic PPTg/LDTg neurons [8]) produces a sustained release of dopamine in the NAc [9]. To investigate whether our neurochemical findings translate to measurable behavioral changes we used the CPP paradigm. In addition, lower doses (10 and 1 pmole) not previously tested for their effect on NAc dopamine levels were investigated for their abilities to modulate cocaine-mediated CPP and VTA-targeted electrically evoked dopamine release in the NAc. The present study is supportive of UIIR activation having behavioral effects that are of key interest to the fields of drugs of abuse and reward-related behaviors.

2. Experimental procedures

2.1. Conditioned place preference

2.1.1. Animals

Male Sprague–Dawley rats (290–340 g) (Charles River, Wilmington, MA) were used for all experiments. Animals were housed in an environmentally controlled vivarium with a 12 h light/dark cycle (lights on at 07:00) with an ambient temperature of ~21 °C. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University at Buffalo, and were in compliance with guidelines set by the National Institute of Health (NIH). All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.1.2. Stereotaxic surgery

Bilateral cannulation of the ventral tegmental area (VTA) using a stereotaxic apparatus (Steolting Co., Wood Dale, IL) was performed under Ketamine HCl (Ketaset, Fort Dodge, IA)/Xylazine (Anased, Lloyd, Shenandoah, IA) (65 mg/5 mg/kg) anesthesia. Stereotaxic coordinates of subjects' bregma were recorded, and drill sites for guide cannula (Plastics One, Roanoke, VA) were calculated [anteroposterior (AP), −6.5 mm; mediolateral (ML), ±3.2 mm]. Cannula sites were drilled, and three additional holes were made for the placement of anchor screws (Plastics One, Roanoke, VA), one on each skull plate just posterior to bregma, and one posterior to lambda. Anchor screws were secured to the skull, and stereotaxic arms (20°) lowered cannula into holes until dura. From dura, cannula were slowly lowered [dorsoventral (DV), −6.9 mm] and held in place for the application of methyl methacrylate (Co-Oral-Ite Dental Mfg. Co., Diamond Springs, CA). Using this technique the desired placement of the cannual was 1 mm above the VTA with final cannula coordinates of (AP −6.5 mm, ML +/−0.6 mm, DV −7 mm, from bregma [47]; the placement was based on estimates from the in situ radioligand binding to UIIR [10]). Methyl methacrylate head caps were allowed to harden, and screw caps (Plastics One, Roanoke, VA) were inserted into cannula. Following surgery, buprenorphine hydrochloride (0.05 mg/kg; Reckitt Benckiser Pharmaceuticals, Richmond, VA) was administered subcutaneously. Subjects were also treated with a 6.0 mL injection (SC) of lactated ringers (Hospira, Lake Forest, IL) for hydration, and an injection (SC) of enrofloxacin (Bayer, Leverkusen, Germany) to prevent infection.

Post-operatively, subjects were individually housed to recover for 5–6 days, and handled daily in preparation for the behavioral testing.

2.1.3. Behavioral assay

Conditioned place preference (CPP) boxes (39 cm × 39 cm) were designed and constructed in-house to suit the experimental environment. Boxes were constructed of plexiglass with two equivalent compartments, one with black and white horizontally stripped walls with metal grid flooring and lemon scent, and the other with black and white dotted walls with metal bar flooring and banana scent. Boxes were placed in infrared locomotor capture apparatus (OMNITECH Electronics, Columbus, OH) controlled by Fusion software. All 30 min sessions were run during the day (09:00–15:00). Dose groups were randomized throughout the day to mitigate any time of day effects.

On day 1 (pretest), partitions with cutout doorways were inserted into boxes allowing subjects to freely explore both compartments; amount of time spent in each compartment was