Progress in Neuro-Psychopharmacology & Biological Psychiatry 44 (2013) 248–256

Contents lists available at SciVerse ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

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

Angiotensin II's role in sodium lactate-induced panic-like responses in rats with repeated urocortin 1 injections into the basolateral amygdala
Amygdalar angiotensin receptors and panic

Philip L. Johnson [a,b], Tammy J. Sajdyk [a], Stephanie D. Fitz [a], Mathew W. Hale [c], Christopher A. Lowry [d], Anders Hay-Schmidt [e], Anantha Shekhar [a,*]

[a] Department of Anatomy & Cell Biology, Indiana University School of Medicine, Indianapolis, IN, United States
[b] Institute of Psychiatric Research Departments of Psychiatry, Indiana University School of Medicine, Indianapolis, IN, United States
[c] School of Psychological Science, La Trobe University, Melbourne, Vic, Australia
[d] Department of Integrative Physiology and Center for Neuroscience, University of Colorado Boulder, Boulder, CO, United States
[e] Department of Neuroscience and Pharmacology, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark

ARTICLE INFO

Article history:
Received 24 November 2012
Received in revised form 19 February 2013
Accepted 26 February 2013
Available online 21 March 2013

Keywords:
Amygdala
Angiotensin
Anxiety
Circumventricular organ
Panic
Saralasin

ABSTRACT

Rats treated with three daily urocortin 1 (UCN) injections into the basolateral amygdala (BLA; i.e., UCN/BLA-primed rats) develop prolonged anxiety-associated behavior and vulnerability to panic-like physiological responses (i.e., tachycardia, hypertension and tachypnea) following intravenous infusions of 0.5 M sodium lactate (NaLac, an ordinarily mild interoceptive stressor). In these UCN-primed rats, the osmosensitive subfornical organ (SFO) may be a potential site that detects increases in plasma NaLac and mobilizes panic pathways since inhibiting the SFO blocks panic following NaLac in this model. Furthermore, since SFO neurons synthesize angiotensin II (A-II), we hypothesized that the SFO projects to the BLA and releases A-II to mobilizing panic responses in UCN/BLA-primed rats following NaLac infusions. To test this hypothesis, rats received daily bilateral injections of UCN or vehicle into the BLA daily for 3 days. Five to seven days following the intra-BLA injections, we microinjected either the nonspecific A-II type 1 (AT1r) and 2 (AT2r) receptor antagonist saralasin, or the AT2r-selective antagonist PD123319 into the BLA prior to the NaLac challenge. The UCN/BLA-primed rats pre-injected with saralasin, but not PD123319 or vehicle, had reduced NaLac-induced anxiety-associated behavior and panic-associated tachycardia and tachypnea responses. We then confirmed the presence of AT1rs in the BLA using immunohistochemistry which, combined with the previous data, suggest that A-II's panicogenic effects in the BLA is AT1r dependent. Surprisingly, the SFO had almost no neurons that directly innervate the BLA, which suggests an indirect pathway for relaying the NaLac signal. Overall these results are the first to implicate A-II and AT1rs as putative neurotransmitter-receptors in NaLac induced panic-like responses in UCN/BLA-primed rats.

1. Introduction

Panic disorder is a severe anxiety disorder characterized by recurrent panic attacks, consisting of pronounced fear and heightened cardiorespiratory responses (DSM-IV, 1994). A unique characteristic of panic disorder patients is their sensitivity to ordinarily mild interoceptive stressors such as intravenous infusions of mildly hypertonic 0.5 M sodium lactate (NaLac) (Cowley et al., 1987; Lapierre et al., 1984; Liebowitz et al., 1985). The pathological sensitivity to interoceptive stressors appears to involve an alteration somewhere in the central neural pathways controlling normal panic responses. The amygdala may be part of this circuitry since abnormalities in this structure have been noted in panic patients (Reiman et al., 1984, 1989; Wiest et al., 2006).

The amygdala plays a critical role in attention mechanisms that facilitate learning and survival in response to important sensory input [see review (LeDoux, 2000)]. Synaptic plasticity changes within the basolateral amygdala (BLA) parallel anxiety-like behavior and are believed to be important in fear-associated memories (Mitra et al., 2005). Preclinical data from our laboratory support the hypothesis that aberrant conditions within the amygdala underlie panic attacks in some cases. For instance, chronic loss of local inhibitory GABAergic tone (Sajdyk and Shekhar, 2000; Shekhar et al., 1999), or three daily injections of urocortin 1 (UCN, a potent corticotropin-releasing factor 1 and 2 receptor agonist) (Rainnie et al., 2004; Sajdyk et al., 1999a)

Abbreviations: UCN, Urocortin 1; BLA, basolateral amygdala; A-II, angiotensin; AT1r, angiotensin type 1 receptor; AT1r, angiotensin type 2 receptor.

* Corresponding author at: Department of Psychiatry, Indiana University Medical Center, 1111 West 10th St., Suite 313, Indianapolis, IN 46202, United States. Tel.: +1 317 274 1246; fax: +1 317 278 4821.
E-mail address: ashekhar@IUPUI.edu (A. Shekhar).

0278-5846/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.pnpbp.2013.02.014

within the BLA produces rats that display a chronic anxiety-like state and are also prone to panic-like responses following intravenous infusions of 0.5 M NaLac.

Previous clinical studies suggest that increased sodium, not lactate or osmotic stress, may be the critical factor for provoking panic attacks in panic disorder patients. For example, i.v. infusions of hypertonic (0.5 M) NaLac, sodium chloride (NaCl) (Peskind et al., 1998), or sodium bicarbonate (Gorman et al., 1989) provoke equivalent panic associated responses in panic disorder patients; and in a hypothalamic rat model of panic vulnerability, i.v. infusions of hypertonic (0.5 M) solutions of NaCl and NaLac resulted in equivalent panic-like behavioral and cardiovascular responses (Molosh et al., 2010). In rodents, the expression of the Na_X channel protein, which is critical for changes in drinking behavior in response to increases in plasma concentrations in Na^+ [i.e., dehydration (Watanabe et al., 2000)], is largely restricted to regions with a reduced blood-brain barrier called circumventricular organs (CVOs) (Hiyama et al., 2004; Watanabe et al., 2000).

Circumventricular organs are believed to play a critical role in relaying osmotic-related signals (e.g., changes in Na^+) from the periphery to the central nervous system (Hochstenbach and Ciriello, 1996). Previously, Shekhar et al. (1999) have demonstrated the importance of the forebrain subfornical organ (SFO) CVO in NaLac's ability to elicit panic-like responses in UCN/BLA-primed rats. The SFO may be especially important in relaying the NaLac signal to the BLA since blocking neuronal transmission in the SFO of UCN/BLA primed rats prevents NaLac-induced anxiety-like and cardiorespiratory responses (Shekhar et al., 1999). The present study was designed to determine the neurochemical substrate underlying this NaLac-SFO-BLA circuit. Since the majority of SFO neurons produce angiotensin (A-II) (Lind et al., 1985a, 1985b; Saavedra and Chevillard, 1982; Saavedra et al., 1982), and centrally acting A-II type 1 receptor (AT1r) antagonists are anxiolytic (Saavedra et al., 2005, 2006), there is reason to believe that A-II may be the critical neuropeptide in this circuit. In order to test this hypothesis, rats were made panic-prone by receiving 3 daily bilateral injections of UCN into the BLA. After another 3-5 days, rats then received bilateral injections of saline vehicle, saralasin (a nonselective A-II receptor antagonist), or PD123319 [a selective A-II type 2 receptor (AT2r) antagonist] into the BLA 30 min prior to i.v. NaLac infusions. Furthermore, the presence of AT1Rs in the BLA was assessed using immunohistochemistry. In light of the presence of A-II-synthesizing neurons in the SFO, and the SFO's ability to 'sense' the NaLac signal, we also determined if neurons in the SFO directly innervate the BLA region, using intra-BLA microinjections of retrograde tracer.

2. Methods and materials

2.1. Animals

All experiments, except for the retrograde tracer experiment, were conducted on adult male Wistar rats (300–325 g), which were purchased from Harlan Laboratories and were housed individually in plastic cages under standard environmental conditions (22 °C; 12/12 light/dark cycle; lights on at 7:00 A.M.) for 7–10 days prior to the surgical manipulations. Food and water were provided *ad libitum*. Animal care procedures were conducted in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals (NIH Publication no. 80-23) revised 1996 and the guidelines of the IUPUI Institutional Animal Care and Use Committee.

For the retrograde tracing experiment, adult male Wistar rats arrived from the vendor (Møllegaarden, Denmark; N = 34) weighing approximately 200 g and were kept in standard laboratory conditions with *ad libitum* tap water and standard rat chow (Altromin, Lage, Germany) and maintained under a photoperiod of 12-h light:12-h dark with lights on at 6:00 AM. The experiment was conducted under the authority of the Animal Core Facility of the Panum Institute, Department of Neuroscience and Pharmacology, The Panum Institute, University of Copenhagen, in accordance with and approved by The Animal Experiments Inspectorate, Ministry of Justice, Denmark and the European Communities Council Directive of 24 November 1986 (86/609/EEC). Care was taken to minimize the number of animals used and their suffering. Experimental data from these rats have been previously reported (Hale et al., 2008a, 2008b)

2.2. Venous catheterization for NaLac infusions

Prior to surgery, rats were anaesthetized by placing them in a closed Plexiglas® box that was connected to an isoflurane system (MGX Research Machine; Vetamic, Rossville IN) and then with a nose cone connected to the same system during the surgery. All rats were fitted with femoral arterial catheters for measurement of mean arterial blood pressure (MAP) and heart rate (HR) and with femoral venous catheters for i.v. infusions, as previously described (Shekhar et al., 1996). Briefly, MAP and HR were monitored by an arterial line attached to a pressure transducer connected to a Beckman R511 Dynograph (Beckman Instruments. Inc., Brea, CA). Windows based DSI DATAQUEST software was used to monitor and record MAP and HR, whereas an indirect measurement of respiration rate (RR) was obtained from normal sinus arrhythmia. For the duration of each experiment, MAP, HR and RR were recorded continuously in freely moving conscious rats. Cardio-respiratory data are expressed as peak changes in MAP or HR. The peak for MAP and HR was defined as the highest value sustained for 1 min or longer.

2.3. Implantation of chronic microinjection cannulae into the BLA and drug injections

Immediately following venous and arterial catheterization, rats were placed into a stereotaxic instrument (Kopf Instruments, Tujunga, CA) with the incisor bar set at −3.3 mm and nose cone connected to the same system during the surgery. Two stainless steel guide cannulae (26 gauge, 10 mm length: Plastics One, Roanoke, VA) were situated into guide cannulae holders fixed onto the stereotaxic arms. The injector was lowered into position of the BLA using coordinates (anterior, −2.1 mm; lateral, ±5.0 mm; ventral, −8.5 mm) according to a standard stereotaxic atlas of the adult rat brain (Paxinos and Watson, 1986). The guide cannulae were secured into place using three 2.4 mm screws anchored into the skull along with cranioplastic cement. Following placement of dummy cannulae into the guide cannulae, rats were removed from the stereotaxic apparatus and allowed to recover for 72 h.

All injections of UCN into the BLA were conducted utilizing microinjection cannulae (33 gauge, Plastics One) that fit into and extended 1 mm beyond the guide cannulae. UCN was administered in 1% bovine serum albumin (BSA) in a total volume of 100 nl per/site. A 10-μl Hamilton syringe was situated on an infusion pump (Harvard Apparatus, Holliston, MA, model PHD 2000) and subsequently connected to the injection cannulae via polyethylene (PE 50) tubing (Fisher Scientific, Pittsburg, PA). Once the injection cannulae were securely placed into the rat, the infusion pump was turned on and set to automatically deliver 100 nl/site over 30 s. Following the injection, the cannulae remained in place for an additional min before being removed. Smooth flow of the solutions via the tip of the injection cannulae was verified before and after each injection to ensure proper drug delivery. UCN was generously provided by Dr. Nick Ling (Neurocrine, San Diego, CA).

2.4. Experimental protocol

After 3 days of recovery from surgeries, baseline reactivity to i.v. NaLac infusions was determined [(i.e., cardiorespiratory responses and social interaction (SI) test immediately following the end of NaLac challenge]). Seventy-two h following BLA cannulations, 6 rats received