Behavioural Brain Research 226 (2012) 41–49

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

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

Research report

Stress-evoked increases in serotonin in the auditory midbrain do not directly result from elevations in serum corticosterone

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ARTICLE INFO

Article history:
Received 8 June 2011
Received in revised form 26 August 2011
Accepted 27 August 2011
Available online 3 September 2011

Keywords:
Auditory
Corticosterone
Inferior Colliculus
Neuromodulation
Serotonin
Stress

ABSTRACT

Neurochemicals such as serotonin convey information about behavioral context to sensory processing. In the auditory system, serotonin modulates the responses of neurons in the inferior colliculus (IC) to acoustic stimuli, including communication vocalizations. Levels of extracellular serotonin in the IC can change rapidly in response to stressful situations such as social challenge and limited movement. Since activation of the hypothalamo–pituitary–adrenal (HPA) axis can influence serotonin in other brain regions, we examined the relationship between serum corticosterone and serotonin release in the IC. We used voltammetry to measure extracellular serotonin in the IC of male CBA/J mice during restriction of movement, a low-intensity restraint stress. Enzyme immunoassay (EIA) was used to measure the concentration of corticosterone circulating in the blood serum as an indicator of the activation of the HPA axis. Changes in serotonin and corticosterone were also compared with behavioral performance. Restriction stress caused increases in serotonin in the IC and circulating corticosterone, and changes in behavior. Changes in serotonin and corticosterone were not correlated with each other across individuals. Individual behavioral performance was correlated with elevations in corticosterone, but not in serotonin. We further explored the relationship between physiological pathways by directly manipulating serum corticosterone. Injections of corticosterone elevated circulating levels beyond normal physiological ranges, but had no effect on serotonin in the IC. These findings suggest that, within the auditory system, serotonin is released during stressful events, but this is a correlate of behavioral arousal, rather than a direct response to elevations in serum corticosterone.

1. Introduction

Exposure to stress induces a broad set of physiological and behavioral changes selected to increase the probability of survival. Perhaps the most well characterized physiological response to stress is the activation of the hypothalamo–pituitary–adrenal (HPA) axis, which results in the secretion of glucocorticoids into the blood stream and the activation of glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs) in tissues throughout the body. These mechanisms ultimately induce suites of metabolic, endocrine, and neural changes that facilitate performance in stressful environments [1–5]. The activation of the HPA axis is variable, such that the magnitude and time course are often correlated with both the exact nature of the stressor and the subsequent behavioral response of the individual [6,7]. The behavioral response to a stressor can also be influenced or facilitated by other physiological changes that are coincident with the activation of the HPA axis, including the alteration of sensory processing [8,9]. In this way, stress-induced changes in sensory processing could have evolutionary consequences by providing the advantage of enhanced perception of salient stimuli in situations of duress, for example. Though the amygdala and prefrontal cortex have been the focus of much of the research in this area, a growing body of evidence suggests that some stress-related changes in perception can occur at earlier stages of sensory processing [10,11].

The relationship between stress and sensory processing is evident in the auditory system. Stress and the subsequent changes in glucocorticoid levels are correlated with changes in the timing and amplitude of auditory evoked potentials in both humans and mice [8,12]. Stress can also induce auditory hypersensitivity

Abbreviations: ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone; CV, coefficient of variation; DOPAC, 3,4-dihydroxyphenylacetic acid; EIA, enzyme immunoassay; GLM, general linear model; GR, glucocorticoid receptor; HPA, hypothalamo–pituitary–adrenal axis; IC, inferior colliculus; i.p., intraperitoneal; MR, mineralocorticoid receptor; OCT3, organic cation transporter 3; s.c., subcutaneous; s.e.m., standard error of the mean.

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doi:10.1016/j.bbr.2011.08.042

[13] or influence auditory pathologies such as tinnitus or Meniére's disease [14,15]. Further, exposure to restraint stress prior to acoustic trauma has a protective effect against noise-induced hearing loss [16]. These impacts of stress on the auditory system may be due to direct or indirect effects of the activation of the HPA axis. MRs are found in the inner ear [17,18] and GRs are distributed throughout the peripheral and central auditory system [19] but the activation of GRs elsewhere in the brain can also have strong effects on multiple modulatory systems, including the serotonergic system, to facilitate a coordinated response to stress at the sensory level [2].

In the inferior colliculus (IC), a midbrain auditory nucleus, the response properties of auditory neurons are influenced by the presence of serotonin [20,21]. Serotonin receptors modulate aversive behavior produced by direct stimulation of the IC [22,23]. Moreover, extracellular serotonin in the IC increases to varying degrees when mice are exposed to noise, restriction of movement or social intrusion, all potentially stressful stimuli [24,25]. It is unclear, however, whether serotonergic increases in the IC directly result from activation of the HPA axis. The serotonergic response in the IC may be part of a separate pathway, independent of the HPA axis, which modulates auditory processing in a variety of contexts that are not necessarily stressful [25–27].

To investigate the relationship between indicators of global physiological stress and the local serotonergic response to the same stressors in the IC, we exposed mice to restriction stress and monitored their physiological and behavioral responses. Restriction of movement, a non-auditory stressor, was used in these experiments to avoid any potential confounding effects of acoustic overstimulation. In addition, in our hands, restraint stress has induced larger and more reproducible increases in serotonin in the IC than acoustic stimulation [25]. We measured circulating levels of corticosterone in the serum before and after restriction as a measure of the activation of the HPA axis, a commonly used indicator of the intensity of a stressor. We also monitored changes in serotonin in the IC during restriction using *in vivo* voltammetry. An individual's corticosterone and serotonergic responses to restriction of movement were compared to each other and to behavior to determine whether any of these responses were correlated. Further, we examined the possibility of a causal link between elevations in peripheral levels of corticosterone and changes in serotonin in the IC by artificially elevating levels of circulating corticosterone via subcutaneous (s.c.) injection.

2. Materials and methods

2.1. Animals

Data were obtained from 15 male CBA/J mice (*Mus musculus*, Jackson Laboratory, Bar Harbor, ME, USA). This strain shows behavioral and physiological responses to stress that are comparable to other strains of mice [28]. Mice ranged in age from 8 to 35 weeks and averaged 21 ± 2.2 (s.e.m.) weeks old. Mice were housed individually on a 14/10 light-dark cycle and supplied with food and water available *ad libitum*. All protocols were approved by the Bloomington Institutional Animal Care and Use Committee.

2.2. Voltammetry

2.2.1. Carbon fiber electrode construction

Carbon fiber electrodes were prepared from pulled glass capillary tubes. The glass tip was broken and a single carbon fiber (11 μm in diameter, Thornell P25; Cytec Industries Inc., West Paterson, NJ) was threaded through the tube until it protruded from the end of the glass 100–150 μm. The carbon fiber was sealed in place with an epoxy resin (Miller-Stephenson Chemical Company, Inc.; Danbury, CT) and soldered to a length of wire that protruded from the back of the electrode using a low melting-temperature bismuth alloy (Small Parts; Miramar, FL).

Carbon fiber electrodes were electrically and chemically treated prior to use in order to increase the sensitivity to and selectivity for serotonin [24]. To increase sensitivity and to separate the electrochemical signal of serotonin from that of other oxidizable molecules, all electrodes were electrically treated in phosphate–citrate buffered saline. An e-Corder controlled by Chart software (EDAQ; Denistone East, Australia) was used to produce a triangular wave form (70 Hz, 0–3 V vs. calomel auxiliary electrode) for 40 s followed by a 1.5 V constant potential for 10 s, a −0.5 V constant potential for 5 s and a 1.5 V constant potential for 8 s. All electrodes were then coated with Nafion, an ion exchange resin, by dipping them in a 5% solution of Nafion (Sigma–Aldrich, St. Louis, MO) 3 times and allowing them to air dry for 3 min between each dip. Nafion coating increases electrode specificity for serotonin; this was confirmed by testing each electrode prior to use in a solution of 50 μM ascorbic acid, 5 μM 3,4-dihydroxyphenylacetic acid (DOPAC) and 1 μM serotonin (Sigma–Aldrich, St. Louis, MO). Electrodes were used in experiments if they measured a serotonin signal at approximately +250 mV (vs. Ag/AgCl) and showed little or no response to DOPAC and ascorbic acid.

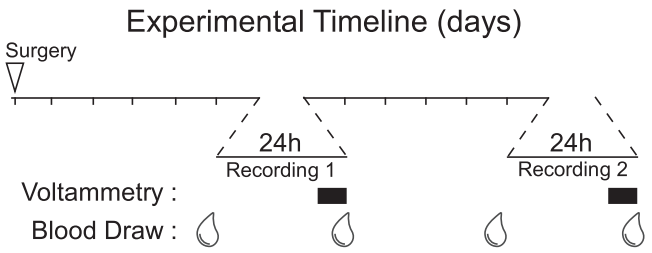

Fig. 1. Experimental timeline. Coordinated measurements of serotonin during limited movement (black bars) and serum corticosterone (drop symbols) were performed twice over a 2-week period in the same mice following the implantation of voltammetric hubs (inverted triangle). Baseline blood samples were taken 24 h prior to samples after the restriction of movement.

2.2.2. Surgery

Head stages were surgically mounted on the skull overlying the IC to permit voltammetric recording in behaving mice (Fig. 1, inverted triangle). Prior to surgery, mice received metacam [1 mg/kg, s.c.]. After at least 1 h, mice were anesthetized with an intraperitoneal (i.p.) injection of ketamine (120 mg/kg) and xylazine (5 mg/kg). Anesthetic state was determined by lack of response to tail pinch.

Once mice were fully anesthetized, the hair on top of the head was removed with depilatory cream and mice were placed in a stereotaxic device for aseptic surgery (Stoelting; Wood Dale, IL). An incision was made to expose the skull from bregma to the musculature of the neck. Bregma and lambda were placed in the horizontal plane and holes approximately 1.5 mm in diameter were drilled over the left and right IC (1.1 mm posterior, 1.6 mm lateral to lambda). Custom-made Teflon hubs [29], designed to mate with micro-drives containing the recording and reference electrodes, were placed above each hole at an angle of 15° from vertical. Hubs were secured to the skull with two stainless steel bone screws (PlasticsOne Inc.; Roanoke, VA) and dental cement. Between experiments, the hubs were plugged with custom-made Teflon screws. On the day following surgery, mice received metacam (1 mg/kg, s.c.). Mice were allowed to recover from surgery for at least 7 days prior to recording.

2.2.3. Recording

Immediately prior to voltammetric recording, mice were anesthetized with ketamine (90 mg/kg) and xylazine (1 mg/kg) for the placement of the electrodes. Both the carbon fiber recording electrode and Ag/AgCl reference electrodes were mounted in custom-made, Teflon microdrives that screwed into the hubs mounted on the skull. After the microdrives were secured in the hubs, the recording electrode was lowered into the IC on one side of the brain and the reference electrode was lowered until it came into contact with the cerebrospinal fluid above the IC on the other side of the brain. Immediately after electrode placement, mice were returned to their home cage, which was then placed in a Faraday chamber. Mice were allowed to recover from anesthetic on a 10 cm^2 piece of laboratory tissue in order to separate them from the bedding material and were considered to have recovered once they voluntarily walked off of the tissue. A light-weight, flexible tether connected the recording and reference electrodes to a bipotentiostat (EI-400; Cypress Systems, Chelmsford, MA) through an electric swivel (PlasticsOne Inc.; Roanoke, VA) which allowed the mouse to move freely around the cage. Voltammetric recording began as soon as the electrodes were connected to the tether and were performed continuously throughout the recording session. A staircase waveform (−300 mV to +600 mV to −300 mV, 10 mV steps, 30 mV/s, 1 min duration) was applied with 5 min between each waveform, so that one serotonin measurement was made every 6 min. All manipulations were performed more than 1 h after the mice recovered from anesthesia. Throughout the recording session, the cage was surrounded by an opaque barrier, 40 cm tall, in order to decrease interference from external visual stimuli and prevent mice from escaping.

Voltammetric recording took place in two sessions, one in each IC, with at least a 7-day recovery period between sessions (Fig. 1, black boxes). Whether the first session recorded from the left or right IC was chosen randomly. For each mouse, both sessions occurred at the same time of day, from 09:00 h to 12:00 h or 13:00 h to 16:00 h. Over the two sessions, mice were exposed to a restriction stressor and a control manipulation, or a corticosterone injection and a control injection. Whether the control manipulation occurred in the first or second recording session was determined randomly. In order to facilitate the rapid collection of blood after the