The effect of short moderate stress on the midbrain corticotropin-releasing factor system in a macaque model of functional hypothalamic amenorrhea

Cynthia L. Bethea, Ph.D.,[a,b,c,d] Kenny Phu, B.S.,[a] Arubala P. Reddy, Ph.D.,[a] and Judy L. Cameron, Ph.D.[e]

[a] Divisions of Reproductive Sciences, and [b] Neuroscience, Oregon National Primate Research Center, Beaverton, Oregon; [c] Departments of Behavioral Neuroscience, and [d] Obstetrics and Gynecology, Oregon Health and Science University, Portland, Oregon; and [e] Department of Psychiatry, University of Pittsburgh, Pittsburgh, Pennsylvania

Objective: To study the effect of moderate stress on corticotropin-releasing factor (CRF) components in the serotonergic midbrain region in a monkey model of functional hypothalamic amenorrhea.
Design: After characterization of stress sensitivity, monkeys were moved to a novel room and given 20% less chow for 5 days before euthanasia.
Setting: Primate research center.
Animal(s): Female cynomolgus macaques (*Macaca fascicularis*) characterized as highly stress resilient (HSR, n = 5), medium stress resilient (n = 4), or stress sensitive (SS, n = 4).
Intervention(s): Five days of diet in a novel room with unfamiliar conspecifics.
Main Outcome Measure(s): Density of CRF axons in the serotonergic dorsal raphe nucleus; the number of urocortin 1 (UCN1) cells; the density of UCN1 axons; the expression of CRF receptor 1 (CRF-R1) and CRF-R2 in the dorsal raphe nucleus.
Result(s): The CRF innervation was higher in HSR than in SS animals; UCN1 cell number was higher in HSR than in SS animals and UCN1 axon bouton density was not different; all opposite of nonstressed animals. The CRF-R1 was not different between the sensitivity groups, but CRF-R2 was higher in HSR than in SS animals. The relative expression of CRF-R1 and CRF-R2 was similar to nonstressed animals.
Conclusion(s): The HSR animals respond to stress with an increase in CRF delivery to serotonin neurons. With stress, UCN1 transport decreases in HSR animals. The CRF receptor expression was similar with or without stress. These changes may contribute to resilience in HSR animals. (Fertil Steril® 2013;100:1111–21. ©2013 by American Society for Reproductive Medicine.)
Key Words: Stress, resilience, ovulation, amenorrhea, serotonin, CRF, macaques

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/betheacl-stress-crf-macaques/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

Exposure to stressful stimuli can lead to a variety of secondary diseases such as anxiety, depression, cardiovascular disease, and immune hyperactivity (1, 2). Reproductive dysfunction has been recently added to this growing list of stress-related disorders (3, 4). Functional hypothalamic amenorrhea (FHA) is a disorder that occurs in women with no identifiable organic cause who tend to score higher than average on psychometric tests for stress; who diet but do not qualify for anorexia or other eating disorders; who exercise regularly; and who have no menstrual cycles for more than 6 months (5–7). Functional hypothalamic amenorrhea is also called stress-induced amenorrhea, and it is clear that some individuals are very sensitive to stressors, whereas others are stress resilient. About 30% of female amenorrhea is diagnosed as FHA or stress-induced amenorrhea (8).

Received March 8, 2013; revised and accepted May 31, 2013; published online July 10, 2013.
C.L.B. reports study funding and travel support from National Institutes of Health/National Institute of Child Health and Human Development grant. K.P. has nothing to disclose. A.P.R. has nothing to disclose. J.L.C. reports study funding and travel support from National Institutes of Health/National Institute of Child Health and Human Development grant.
Supported by National Institutes of Health grants HD62618 to J.L.C. and C.L.B., P30-NS06180 to Dr. Sue Aicher, and P51 OD011092 for the operation of ONPRC.
Reprint requests: Cynthia L. Bethea, Ph.D., Oregon National Primate Research Center, 505 NW 185 Avenue, Beaverton, Oregon 97006 (E-mail: betheac@ohsu.edu).

Fertility and Sterility® Vol. 100, No. 4, October 2013 0015-0282/$36.00
Copyright ©2013 American Society for Reproductive Medicine, Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.fertnstert.2013.05.052

Effective stress management and removal of metabolic stresses result in the restoration of fertility in most patients (9–11).

We have developed an experimental nonhuman primate model of FHA in which mild psychosocial stress combined with a mild diet, plus or minus a moderate exercise regimen, leads to suppression of reproductive function to different degrees that reverses upon stress removal (3, 12, 13). Female cynomolgus monkeys are either [1] highly stress resilient (HSR) and maintain normal menstrual cyclicity when exposed to two cycles of combined stress, or [2] medium stress resilient (MSR) and ovulate in the first stress cycle, but not in the second stress cycle, or [3] stress sensitive (SS) and become anovulatory as soon as stress is initiated, similar to women with FHA (3, 12, 13). Two major central nervous systems are thought to govern stress responses (i.e., the corticotropin-releasing factor [CRF] and serotonin system) (13, 14).

A reciprocal relationship between the CRF and serotonin system exists (14). The dorsal and median raphe nuclei send serotonergic projections to the forebrain and diencephalon, including the paraventricular nucleus of the hypothalamus (PVN) (15, 16) and there are CRF projections to the raphe nuclei (17). In contrast with rodent studies, our observations suggest that serotonin inhibits PVN-CRF production in primates (13, 18). Dysfunction of the CRF and serotonin system is common in mood and anxiety disorders and both play critical roles in the stress response (19–22). In addition, the CRF and serotonin systems provide input into the hypothalamic-pituitary-gonadal axis and therefore may affect reproductive potential (23, 24). We have previously demonstrated that there are pivotal differences between HSR and SS animals in functional aspects of the serotonin and CRF systems. The SS animals had lower release of serotonin after a fenfluramine challenge (25), and lower gene expression of tryptophan hydroxylase 2, rate limiting enzyme for serotonin synthesis, serotonin reuptake transporter, serotonin receptor, subtype 1A, and fifth ewing variant (Fev; serotonin developmental master gene) in the serotonergic dorsal raphe nucleus compared with HSR animals (26, 27). With regard to the CRF system, SS animals had higher CRF gene expression in the PVN and denser CRF axon staining in the serotonergic raphe nucleus and noradrenergic locus ceruleus than in HSR animals (18, 28, 29).

We recently reported that after 5 days of moderate stress, the relative expression of the serotonin-related genes was similar to nonstressed conditions. That is, HSR animals still had higher expression of tryptophan hydroxylase 2, rate limiting enzyme for serotonin synthesis, serotonin reuptake transporter, and serotonin receptor, subtype 1A messenger RNAs in the dorsal raphe than SS animals. In the locus ceruleus, HSR animals had denser serotonin axon innervation than SS animals, which was also the same with or without stress (29). Together, the observations suggested that the serotonin system did not markedly change with 5 days of moderate stress, but it continued to reflect stress sensitivity. However, the CRF innervation of the locus ceruleus reversed with stress. In the absence of stress, CRF axon density was lower in HSR than in SS animals. In contrast, after 5 days of moderate stress, CRF innervation of the locus ceruleus was higher in HSR than in SS animals. This study continues the investigation of the CRF in the raphe region. Here we report the expression of dorsal raphe CRF components, CRF, urocortin 1 (UCN1), CRF receptor 1 (CRF-R1), and CRF-R2 in HSR, MSR, and SS animals after 5 days of moderate stress.

MATERIALS AND METHODS

Animals and Treatments

This experiment was approved by the Institutional Animal Care and Use Committee (IACUC) of the Oregon National Primate Research Center. It was conducted in accordance with the 2011 Eighth Edition of the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

Fifteen adult female cynomolgus monkeys (*Macaca fascicularis*) were used. The animals were 7–9 years of age with no prior pregnancies. The animals were imported and immediately housed in single cages in the same room. Cynomolgus macaques form social hierarchies in grouped housing with subordinates receiving more aggression. With single cages, the rank is not a variable. They are the same group of animals used in previous publications examining activity of the hypothalamic-pituitary-adrenal (HPA) axis in response to mild psychosocial and metabolic stress (30), LH pulses in response to a CRF-R1 antagonist (31), and serotonin gene expression (32). Housing, diet, food intake, and daily vaginal swabs for detecting menstruation have been described previously (33, 34). The monkeys were similar in weight, and there were no body weight changes throughout the characterization of stress sensitivity (30).

Assessment of Stress Sensitivity

For each monkey, sensitivity of the reproductive axis to stress was categorized by assessing changes in menstrual cycle length, ovulation, and reproductive hormone secretion when monkeys were exposed to a mild psychosocial and metabolic stressor. The stressor consisted of moving the monkeys to a novel room surrounded by unfamiliar conspecifics and reducing the available chow by 20%, as described previously (13). This study was performed after each monkey had been living in its home cage surrounded by familiar monkeys for several months. Monkeys that menstruated within 38 days subsequent to the initiation of stress were moved for a second stress cycle and remained on 20% lower calorie intake (13). Monkeys that did not mense were not moved a second time.

Animals were categorized as HSR if they presented a normal ovulatory menstrual cycle (25–38 days in length, peak E_2 >200 pg/mL in follicular phase, peak P >2 ng/mL in luteal phase) in stress cycle 1 and again in stress cycle 2. The MSR animals were defined as those animals that presented with a normal ovulatory menstrual cycle in response to stress cycle 1, but failed to mense by day 38 of stress cycle 2. Animals that immediately suppressed normal menstrual cyclicity upon exposure to stress (i.e., they failed to ovulate or mense within 60 days) were categorized as SS. Animals that exhibited disrupted menstrual cycles during this characterization of stress sensitivity (i.e., MSR and SS

Download English Version:

https://daneshyari.com/en/article/6179056

Download Persian Version:

https://daneshyari.com/article/6179056

Daneshyari.com