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Review

Effects of citalopram on serotonin and CRF systems in the midbrain of primates with differences in stress sensitivity☆

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ABSTRACT

This chapter reviews the neurobiological effects of stress sensitivity and s-citalpram (CIT) treatment observed in our nonhuman primate model of functional hypothalamic amenorrhea (FHA). This type of infertility, also known as stress-induced amenorrhea, is exhibited by cynomolgus macaques. In small populations, some individuals are stress-sensitive (SS) and others are highly stress-resilient (HSR). The SS macaques have suboptimal secretion of estrogen and progesterone during normal menstrual cycles. SS monkeys also have decreased serotonin gene expression and increased CRF expression compared to HSR monkeys. Recently, we found that CIT treatment improved ovarian steroid secretion in SS monkeys, but had no effect in HSR monkeys. Examination of the serotonin system revealed that SS monkeys had significantly lower Fev (fifth Ewing variant, rodent Pet1), TPH2 (tryptophan hydroxylase 2), 5HT1A autoreceptor and SERT (serotonin reuptake transporter) expression in the dorsal raphe than SR monkeys. However, CIT did not alter the expression of either Fev, TPH2, SERT or 5HT1A mRNAs. In contrast, SS monkeys tended to have a higher density of CRF fiber innervation of the dorsal raphe than HSR monkeys, and CIT significantly decreased the CRF fiber density in SS animals. In addition, CIT increased CRF-R2 gene expression in the dorsal raphe. We speculate that in a 15-week time frame, the therapeutic effect of S-citalopram may be achieved through a mechanism involving extracellular serotonin inhibition of CRF and stimulation of CRF-R2, rather than alteration of serotonin-related gene expression.

Contents

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Exposure to stressful stimuli can lead to a variety of secondary diseases such as anxiety, depression, cardiovascular disease, and immune suppression (McEwen, 2002). Reproductive dysfunction has been recently added to this growing list of stress-related disorders (Xiao et al., 1999; Cameron, 2000). Clinically, one of the most common forms of infertility is stress-induced infertility, known as functional hypothalamic amenorrhea (FHA; (Berga and Loucks, 2006)). FHA is defined as a sustained absence of normal menstrual cycles despite healthy reproductive organs and the capability of showing normal physiologic functioning (Reifenstein, 1946). International estimates indicate a 9% prevalence of infertility of 12 months (Boivin et al., 2007) and approximately 30% of women presenting at infertility clinics in the United States are diagnosed with functional hypothalamic amenorrhea (FHA), which is lately called stress-induced amenorrhea (Reindollar et al., 1986; Marcus et al., 2001; Berga and Loucks, 2006). Psychometric testing has shown that women with FHA report an increased amount of psychosocial stress in their lives compared to control populations, or women with other forms of infertility, despite experiencing comparable numbers of stressful life events (Giles and Berga, 1993; Fioroni et al., 1994; Marcus et al., 2001). Women with FHA tend to be normal weight and carefully watch their food intake, scoring higher on eating disorder inventories than a control population, but not in the range of having eating disorders (Laughlin et al., 1998; Marcus et al., 2001). They also tend to exercise on a regular basis, often to control life stress (Berga and Girton, 1989; Giles and Berga, 1993; Berga et al., 2003). Thus, there is a body of knowledge indicating that FHA, as well as other forms of stress-induced reproductive dysfunction, results from exposure to a combination of common psychosocial and metabolic life stresses (Williams et al., 2007). Clearly, not all women who experience these everyday life stresses develop fertility problems, suggesting that there is a range of sensitivity to developing stress-induced reproductive dysfunction in normal women.

We have developed an experimental nonhuman primate model of FHA in cynomolgus macaques (*Macaca fascicularis*), which like women, display monthly menstrual cycles with no seasonal variation. Our stress paradigm was modeled on the mild levels of psychosoical stress and the diet and exercise habits reported by women with FHA (Biller et al., 1990; Berga et al., 1997; Marcus et al., 2001; Williams et al., 2007). Indeed, we found that mild psychosocial stress (relocation) combined with a mild diet and/or a moderate exercise regimen suppresses reproductive function in about a third of cynomolgus macaques, which reverses upon stress removal (Williams et al., 1997, 2007; Cameron, 2000; Bethea et al., 2005a).

Fig. 1 illustrates the paradigm employed to determine stress sensitivity. Cynomolgus monkeys are monitored until regular menstrual cycles are present. When the paradigm is started, the animals are observed through two control cycles during which the animal first observes another monkey on the treadmill and then is placed on the treadmill and learns to run. At the beginning of the third cycle, the animal is moved to another room with unfamiliar animals (psychosocial stress), placed on 80% of ad libitum monkey chow and allowed to run 5 days per week. After 30 days, the animal is moved again to another room with unfamiliar conspecifics and continues the diet and exercise. Following 30 days in the second room, the animal is returned to its original room and the diet and exercise are ceased.

Using this monkey model, we find that some individuals are sensitive to stress-induced suppression of reproductive hormone secretion, while others are stress-resilient. We designate animals 'highly stress-resilient' (HSR) if they maintain normal menstrual cyclicity when exposed to two cycles of combined stress; or medium stress-resilient (MSR) if they are ovulatory in the first stress cycle, but anovulatory in the second stress cycle, or stress-sensitive (SS) if they become anovulatory as soon as stress is initiated. These designations are straightforward, but they apply only to our model of FHA at this time. We have shown that compared to HSR animals, the MSR + SS animals have slightly higher daytime cortisol levels and show a larger increase in cortisol when stressed, which is blocked by antalarmin (Herod et al., 2011a,b).

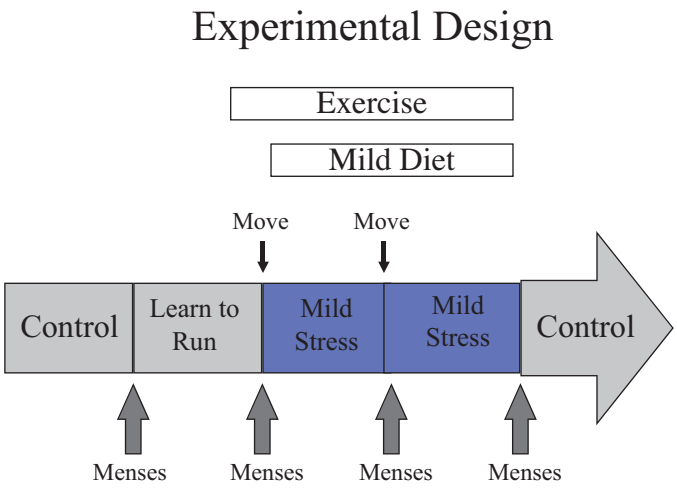

Fig. 1. Schematic diagram of experimental design.

Two major neural systems that are involved in the mediation of stress are (1) the serotonin system and (2) the corticotropin releasing factor (CRF) system. Underpinning serotonin neurotransmission is the expression of genes that determine synthesis, uptake, degradation and autoreceptors. Fev is an ETS domain transcription factor that determines whether a neuron is serotonergic, and functions specifically in the differentiation and maintenance of serotonin neurons (Hendricks et al., 1999, 2003). Tryptophan hydroxylase (TPH2) is the rate limiting enzyme in serotonin synthesis; the serotonin reuptake transporter (SERT) governs the reuptake of serotonin from the synapse and beyond; monoamine oxidases A and B (MAO-A, MAO-B) catalyze the degradation of serotonin and other biogenic amines; and the 5HT1A autoreceptor decreases serotonin neuronal excitation. Dysfunction of serotonin-related gene products has been documented in patients with depression, anxiety or impulse disorders (Mann et al., 2001; Arango et al., 2002) and in animal models of depression, anxiety and impulsive behavior (Champoux et al., 2000). Individual polymorphisms in these genes have also been linked to affective disorders in humans (Arango et al., 2003; Souery et al., 2003; D'Souza and Craig, 2008). In animal models, a number of these polymorphisms interact with the environment, called gene × environment interactions, which in turn appear to produce alterations in behavior resembling neuropsychiatric disorders (Newman et al., 2005).

In many stressful situations the hypothalamic–pituitary–adrenal (HPA) axis becomes activated with increases in CRF, ACTH and cortisol, and CRF may inhibit GnRH release (Williams et al., 1990; Wang and Millam, 1999; Dobson et al., 2003). The CRF system has been implicated in the etiology of depression (Morimoto et al., 1993; Weiss et al., 1994; Nemeroff, 2004b), and a stress-induced elevation in corticotropin releasing factor (CRF) in the hypothalamic paraventricular nucleus (PVN) is thought to underlie the hyperactivity of the HPA axis in depression (Holsboer, 1999; Keck and Holsboer, 2001; de Kloet et al., 2005). In clinical studies, individuals with depression, anxiety or suicide exhibited more CRF neurons in the hypothalamus than normal individuals (Raadsheer et al., 1994; Bao et al., 2005). Moreover, CRF neurons and fibers are found in numerous limbic structures