Hormones and Behavior 55 (2009) 41–49

Contents lists available at ScienceDirect

Hormones and Behavior

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

The interactive effect of the cholinergic system and acute ovarian suppression on the brain: An *f*MRI study

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ARTICLE INFO

Article history:
Received 22 May 2008
Revised 4 August 2008
Accepted 7 August 2008
Available online 28 August 2008

Keywords:
Ovarian hormones
Estrogen
Cholinergic system
Scopolamine
*f*MRI
Memory

ABSTRACT

Recent evidence suggests that loss of ovarian function following ovariectomy is a risk factor for Alzheimer's disease (AD); however, the biological basis of this risk remains poorly understood. We carried out an *f*MRI study into the interaction between loss of ovarian function (after Gonadotropin Hormone Releasing Hormone agonist (GnRHa) treatment) and scopolamine (a cholinergic antagonist used to model the memory decline associated with aging and AD). Behaviorally, cholinergic depletion produced a deficit in verbal recognition performance in both GnRHa-treated women and wait list controls, but only GnRHa-treated women made more false positive errors with cholinergic depletion. Similarly, cholinergic depletion produced a decrease in activation in the left inferior frontal gyrus (LIFG; Brodmann area 45) – a brain region implicated in retrieving word meaning – in both groups, and activation in this area was further reduced following GnRHa treatment. These findings suggest biological mechanisms through which ovarian hormone suppression may interact with the cholinergic system and the LIFG. Furthermore, this interaction may provide a useful model to help explain reports of increased risk for cognitive decline and AD in women following ovariectomy.

Introduction

Ovariectomy has been reported to be a significant risk factor for the development of memory problems and Alzheimer's Disease (AD) in later life (Nee and Lippa, 1999; Rocca et al., 2007). The cognitive risks of ovariectomy are particularly evident in women who had surgery before natural menopause and did not receive estrogen therapy soon after surgery (Rocca et al., 2007). Early menopause, whether due to surgery or natural menopause, has also been associated with greater longitudinal declines in cognitive function later in life (McLay et al., 2007). In clinical studies, surgical and pharmacological suppression of ovarian hormones produces deficits in verbal memory that are reversed following early treatment with estrogen therapy (Sherwin, 1988; Sherwin and Tulandi, 1996). Similarly, in basic science studies, ovariectomy in female rats produces declines in memory on a delayed matching-to-position (DMP) task, and these are reversed following early treatment with estrogen (Gibbs, 2000). Further, animal studies suggest that the cholinergic system plays a critical role in mediating estrogen-related changes in memory on the DMP. For example, basal forebrain cholinergic neurones have been reported to be necessary to observe estrogen-related enhancements in performance (Gibbs, 2002; Gibbs, 2007). Also estrogen enhances acetylcholine release (Gabor et al., 2003) and improves memory performance following acetylcholine release in the hippocampus and frontal cortex (Gibbs et al., 2004). Finally, estrogen has been reported to attenuate the cognitive and behavioral effects of anti-cholinergic drug induced impairments in healthy post menopausal women (Dumas et al., 2006; Dumas et al., 2008). Thus there is growing evidence from basic and clinical studies that acute suppression of ovarian hormones affects memory. However there have been few studies in humans examining biological mechanisms that might explain this relationship and nobody has investigated the interaction of ovarian suppression and cholinergic function.

Gonadotropin Hormone Releasing Hormone agonists (GnRHa) produce an acute 'pseudo' menopause by pharmacologically suppressing ovarian hormone production. GnRHa studies provide a valuable way to study the behavioral and functional effects of ovarian hormone

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doi:10.1016/j.yhbeh.2008.08.008

suppression. Hence, we recently used GnRHa as a model to study the effects of acute loss of ovarian function on verbal episodic memory as deficits in this domain have been most consistently associated with preclinical AD (Backman et al., 2001). We reported that GnRHa significantly decreased activation (i.e. BOLD response; Ogawa et al., 1990) in left prefrontal cortex, and particularly the left inferior frontal gyrus (LIFG), during successful verbal memory encoding (Craig et al., 2007b). The LIFG has been consistently reported to be central to cognitive processing in language and memory tasks, and is thought to contribute to the processing of an item's semantic attributes (see Gabrieli et al., 1998 for review). In particular LIFG activation during encoding is associated with subsequent memory success (Buckner et al., 2000; Staresina and Davachi, 2006). However, it remains unclear how loss of ovarian function exerts its effect on the encoding of new memories. One possible mechanism of action is via modulation of neurotransmitter systems (such as the cholinergic system) that are sensitive to variation in gonadal hormones, and which affect prefrontal cortical function and memory (see Craig and Murphy, 2007 for review).

Many different lines of research highlight the importance of the cholinergic system in learning and memory (see Hasselmo, 2006 for review). Pharmacological studies in animals (Ainger et al., 1991) and humans (Rusted and Warburton, 1989) have, for example, consistently reported that blockade of muscarinic cholinergic receptors by drugs such as scopolamine impairs the encoding of new memories and, in particular, increases the 'false alarm' rate (Barker et al., 1995; Cohen et al., 1994). Human brain imaging studies have also reported that scopolamine reduces prefrontal cortical perfusion (Honer et al., 1988) and verbal memory associated left prefrontal cortical blood flow (Grasby et al., 1995). Thus the literature suggests that scopolamine, like GnRHa, impedes encoding of memory and has actions at the prefrontal cortex. Furthermore, the memory deficits associated with scopolamine have previously been used as a model for the cognitive decline associated with aging and AD (Martinez et al., 1997).

We therefore carried out an event-related functional magnetic resonance imaging (*f*MRI) study to examine the interaction between GnRHa and scopolamine on verbal memory performance and brain activation at the LIFG. Most women had taken part in an earlier study (Craig et al., 2007b) into the effect of GnRHa on brain function. We hypothesised that scopolamine would impede verbal memory recognition performance and that this would be more profound following GnRHa, particularly with respect to 'false alarms'. We also hypothesised that the impact of scopolamine on encoding-related brain activation would be augmented under conditions of ovarian suppression (i.e. greater attenuation of LIFG activation in women who were administered both GnRHa and scopolamine as compared to those who received scopolamine alone).

Materials and methods

Subjects

We included 26 right-handed young (26–47 y) healthy premenopausal women with benign *Leiomyomata uteri* (i.e. 'fibroids'). All women (a) were prescribed GnRHa (two Zoladex® 3.6 mg implants) as part of their routine clinical management and signed informed consent as per the Ethics Committee Guidelines, (b) were screened to exclude past and/or present psychiatric problems using the Beck Depression and Anxiety Inventories (BDI and BAI respectively) (Beck et al., 1988, 1996) and the Structured Clinical Interview for DSM-IV Axis I and II Disorders (First et al., 1997a, 1997b) and (c) had an IQ score greater than 80 and a Mini Mental State Examination score (Folstein et al., 1983) greater than 27. Women were excluded if they had irregular cycles, a history of alcohol/drug abuse, significant medical/neurological problems, if they were taking regular prescribed medication or if they had any blood test results suggesting significant abnormalities with respect to haematological, endocrine, renal or liver function following routine testing.

Blood collection and endocrine determinants

Blood was collected into tubes without anticoagulant and allowed to clot. Samples were centrifuged for 15 min at 1500 g and serum separated within four hours. Estradiol and progesterone concentrations were quantified using a competitive immunoassays (supplied by Bayer Advia Centaur) using direct chemiluminometric technology [*estradiol intra-assay precision*: level 1 (mean concentration = 242.2 pmol/l), CV = 12.1%, level 3 (mean concentration 642.3 pmol/l), CV = 4%, level 5 (mean concentration = 2377.1 pmol/l), CV = 6.3%; *progesterone intra-assay precision*: level 1 (mean concentration = 12.4 pmol/l), CV = 12.4%, level 3 (mean concentration 22.9 pmol/l), CV = 3.7%, level 6 (mean concentration = 154.9 pmol/l), CV = 2.5%]. Luteinising Hormone (LH) and Follicular Stimulating Hormone (FSH) were quantified using two-site sandwich immunoassays (supplied by Bayer Diagnostics Europe) using direct chemiluminometric technology [*LH intra-assay precision*: level 1 (mean concentration = 4.2 IU/L), CV = 2.3%, level 3 (mean concentration 16.4 IU/L), CV = 2.3%, level 6 (mean concentration = 118.2 IU/L), CV = 2.6*; FSH intra-assay precision*: level 1 (mean concentration = 6.9 IU/L), CV = 2.7%, level 3 (mean concentration 23.4 IU/L) CV = 21.2%, level 6 (mean concentration = 144.3 IU/L), CV = 1.2%].

Stimulus materials

Stimulus lists were created using nouns randomly selected from the MRC psycholinguistic database (http://www.psy.uwa.edu.au/MRCDataBase/uwa_mrc.htm). Initially two lists were created; a 300-word list composed of 'living' nouns (e.g. apple, liver) and a 300-word list composed 'non living' words (e.g. chair, mountain). Words ranged in their written frequency of use between 1 and 30 per million (Kucera and Francis, 1967) and were between four and eight letters in length. These lists were then used to create three 100-word encoding lists (A–C) and six 100-word recognition lists. Lists were matched with respect to word length or frequency of use. Encoding lists were made up of 50 words from the 'living' word lists and 50 words from the 'non living' word list. Each of the two 100-word recognition lists included 50 'old words' from the originally encoded list and 50 'new words' that had not been in the encoded list. The 'new words' included an equal number of 'living' and 'non living' items. Each of the three encoding lists (A–C) therefore had two associated recognition lists, each made up of 50 'old words' from the encoded list and 50 'new words'. An additional 16 words were selected from the word pool to create a practice list for the study task.

Encoding task

Subjects were asked to decide whether words presented to them represented a 'living' or 'non living' object by moving a mounted joystick, on their right-hand-side. They were informed that the purpose of this task was to help them learn the words which they would later be asked to recognize. At each visit women were presented with 100 words, each word for a period of 2000 ms, with an inter-stimulus interval of 2000 ms.

Recognition task

The recognition memory test consisted of the 100 'old words', that had previously been presented, together with 100 'new words' (lures) divided into two lists of 100 words presented 5 minutes apart. For each word volunteers had to decide whether they had seen the word before during the experiment by moving the mounted joystick, on