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Relationships between rapid changes in local aromatase activity and estradiol concentrations in male and female quail brain

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ABSTRACT

Estradiol-17β (E_2) synthesized in the brain plays a critical role in the activation of sexual behavior in many vertebrate species. Because E_2 concentrations depend on aromatization of testosterone, changes in aromatase enzymatic activity (AA) are often utilized as a proxy to describe E_2 concentrations. Utilizing two types of stimuli (sexual interactions and acute restraint stress) that have been demonstrated to reliably alter AA within minutes in opposite directions (sexual interactions = decrease, stress = increase), we tested in Japanese quail whether rapid changes in AA are paralleled by changes in E_2 concentrations in discrete brain areas. In males, E_2 in the pooled medial preoptic nucleus/medial portion of the bed nucleus of the stria terminalis (POM/BST) positively correlated with AA following sexual interactions. However, following acute stress, E_2 decreased significantly (approximately 2-fold) in the male POM/BST despite a significant increase in AA. In females, AA positively correlated with E_2 in both the POM/BST and mediobasal hypothalamus supporting a role for local, as opposed to ovarian, production regulating brain E_2 concentrations. In addition, correlations of individual E_2 in POM/BST and measurements of female sexual behavior suggested a role for local E_2 synthesis in female receptivity. These data demonstrate that local E_2 in the male brain changes in response to stimuli on a time course suggestive of potential non-genomic effects on brain and behavior. Overall, this study highlights the complex mechanisms regulating local E_2 concentrations including rapid stimulus-driven changes in production and stress-induced changes in catabolism.

Introduction

While testosterone mediates various behavioral and physiological effects, critical to the actions of this steroid in the brain is its conversion into estrogens via aromatization. Estradiol-17β (E_2) synthesized in the brain has been shown to have a critical, often limiting, role in the activation of sexual behavior (e.g. Balthazart et al., 2009a), parental behavior (Trainor and Marler, 2002), aggression (e.g. Trainor et al., 2006), nociception (Evrard and Balthazart, 2004), cognition (Garcia-Segura, 2008) and neuroprotection (Garcia-Segura, 2008). Often the actions of locally synthesized estrogens are mediated by their genomic effects: they bind to specific nuclear receptors that then act as transcription factors (McEwen and Alves, 1999). However, evidence for faster non-genomic action is growing.

As described in Cornil et al. (2006), three main criteria should be identified to demonstrate a non-genomic behavioral action of E_2: 1) a behavioral effect should occur rapidly (within minutes, rather than hours or days), 2) a specific receptor and/or signaling pathway should be present to mediate such rapid effects of E_2, and 3) a rapid endogenous regulation of E_2 availability in the brain should be identified.

As an example of satisfying the first requirement, estrogens acutely affect a variety of physiological and behavioral endpoints including sexual behavior (for reviews, see Cornil et al., 2012; Luine and Frankfurt, 2012; Micevych and Christensen, 2012). For example, sexual behavior in male Japanese quail is rapidly regulated by local changes in brain estrogen concentrations (as reviewed in Balthazart et al., 2009b; Serendynski et al., 2013). For the second requirement, a large number of studies have identified and continue to describe cellular actions of E_2 mediated by membrane receptors for E_2 (e.g. Huang and Woolley, 2012; Srivastava et al., 2011; Woolley, 2007). Not surprisingly, such receptors have been implicated in the control of physiological and behavioral processes such as sexual behavior (Dewing et al., 2007; Kow and Pfaff, 2004; Serendynski et al., 2013) and auditory processing (Remage-Healey, 2012).

The third requirement, the existence of active mechanisms of regulation of local E_2 concentration, is thought to be fulfilled by the discovery of rapid changes of brain aromatase kinetics in response to conditions that mimic fluctuations of neuronal activity (Cornil and Charlier, 2010). Indeed, it has been shown that aromatase activity (AA) can be altered in vitro via protein phosphorylations such that the enzyme's capacity to generate estrogens is changed rapidly and reversibly (Balthazart and Ball, 2006; Charlier et al., 2011a). In addition,

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different external stimuli, such as sexual interactions or exposure to an acute restraint stress, elicit in vivo rapid changes in AA in a way that is nuclei-, sex- and context-specific. These changes are also reversible (de Bournonville et al., 2013; Dickens et al., 2012). Such acute variations in enzymatic activity would thus provide the endogenous source of steroid as well as the anatomical specificity (localized aromatase expression) and temporal resolution (rapid changes in enzymatic activity) enabling the best efficiency for the former two requirements (Cornil et al., 2006; Saldanha et al., 2011).

Experiments aiming to describe rapid fluctuations in local E_2 concentrations often rely on measurements of AA levels as a proxy due to technical limitations. Experiments utilizing microdialysis have begun to directly measure fluctuations in E_2 concentrations in response to environmental stimuli (e.g. Remage-Healey et al., 2012; Remage-Healey et al., 2008). However, so far this technique has only allowed the quantification of E_2 in samples collected over a period of 30 min and thus provides integrated measures of fluctuations over a relatively long period rather than a point sample. In addition, with microdialysis it is impossible to also identify the mechanisms mediating changes in E_2 concentrations (increased synthesis or decreased catabolism) although there is good evidence that such changes are aromatase driven (Remage-Healey et al., 2008). In this study, we sought to assess how AA levels and E_2 concentrations relate to each other at a specific time point in a specific brain region in a shorter time frame.

Two types of stimuli (sexual interactions and acute restraint stress) that have been studied in our laboratory reliably alter AA within minutes in a manner that is brain nuclei and sex specific (Dickens et al., 2012). Interestingly, the directionality of these changes in AA is counter-intuitive. For example, aromatization of testosterone to E_2 in the medial preoptic nucleus (POM) is known to be required for the activation of male sexual behavior (Balthazart et al., 2009a); however, males allowed to sexually interact with a female show decreases in AA in the POM (de Bournonville et al., 2013). In contrast, despite suggestion that acute stress suppresses sexual behavior (Wingfield et al., 1998), male Japanese quail that are exposed to acute stress show an increase in AA in the POM (Dickens et al., 2011). Finally, although females show similar patterns of AA changes in the POM, it is not clear why females would utilize the local aromatization pathway to control their brain E_2 concentrations when E_2 concentration is relatively high in the circulation.

In this experiment, we tested whether rapid changes in AA are reflected by parallel local changes in E_2 concentrations. To test this directly, we used tissue dissected from specific brain regions in specific individuals to measure *both* AA and E_2 within the same sample. We hypothesized that a direct relationship between AA and E_2 concentrations should be present such that AA increases (as expected for the POM/BST of stressed individuals) would correlate with increases in E_2 concentration whereas AA decreases (as expected for the POM/BST of individuals exposed to a sexual partner) would be associated to decreases in E_2 concentrations.

Materials and methods

Birds

A total of 62 quail (*Coturnix japonica*) were used (females, n = 32 and males, n = 30). Birds were randomly divided between three groups: control, copulation, and stress. *Control birds* (n = 10–11 for each sex) were sampled directly after removal from their home cage. *Copulation group* birds (n = 10 for each sex) were allowed to interact sexually for 5 min before being sampled (see below for details). Finally, *stress* group birds (n = 10–11 for each sex) were restrained for 15 min prior to sampling. All experiments were conducted on 10 week-old quail raised from eggs obtained from our laboratory breeding colony. Birds were maintained on a long day photoperiod (16L:8D) and both males and females were kept gonadally intact. Birds were provided with food and water ad libitum. Experiments complied with the Belgian laws on "Protection and Welfare of Animals" and on the "Protection of experimental animals". Animal use protocol (#1027) was approved by the Ethics Committee for the Use of Animals at the University of Liège.

Experimental procedures

All experiments were run between 0830 and 1200 across three experimental days. Individuals assigned to each group were tested in an alternating manner such that timing within the test day and across days was evenly distributed for all treatment groups and sex. Because we were mainly interested in testing how changes in AA directly correspond to local E_2 concentrations, the design of our experiment did not create groups for direct comparison but rather reproduced scenarios that have been thoroughly tested independently (de Bournonville et al., 2013; Dickens et al., 2012; Dickens et al., 2011) and have demonstrated to produce the most consistent changes in AA.

Control group

Birds were removed from their home cage and immediately sacrificed by decapitation. Animals were not anesthetized to minimize potential neurochemical changes and decrease the exposure time to additional stressors. Trunk blood was collected for steroid analysis and brains were rapidly dissected from the skull and snap frozen on dry ice (average time from removal from home cage to ice < 90s). Blood was collected, without heparin, in Eppendorf® tubes and immediately placed at 4 °C. After allowing the blood to clot overnight, the blood was centrifuged for 10 min at 9300 g to separate the serum. Serum was then stored at −80 °C until assayed.

Copulation group

Males were pre-tested seven times in order to give them sexual experience prior to testing. During these tests, males were paired with females also assigned to the copulation group; pairings were different on each day such that males did not see the same female twice, including on the experimental test day. We pre-tested males and females in this group but not the other groups because our prior studies had shown consistent results (decrease in POM AA) for pre-tested individuals (e.g. de Bournonville et al., 2011). In contrast, prior studies with stressed individuals (increase in POM AA), utilized birds that had not been pre-tested (e.g. Dickens et al., 2011). Since we wanted to limit the number of animals, we chose to prioritize testing the direction of the changes induced in AA compared to E_2, effectively choosing consistency with previous studies rather than consistency between groups.

All males achieved at least one full copulation (cloacal contact movement or CCM) prior to the testing day. During the pre-tests and on the experimental day, pairs of males and females were allowed to sexually interact in an arena (60 × 40 × 50 cm; length × width × height) for 5 min during which time behaviors were recorded (as described below). In previous studies (Cornil et al., 2005; de Bournonville et al., 2013), this time frame has given the most consistent decrease in AA in the medial preoptic nucleus (POM) and medial portion of the bed nucleus of the stria terminalis (mBST). These two nuclei were pooled here as the POM/BST. At the end of the behavior test on the testing day, birds were removed from the arena and immediately sacrificed. Sample collection and processing continued as described for control birds.

Stress group

Birds were restrained for 15 min in a metal cylinder (10 cm diameter × 16 cm length) that had been used in previous experiment (see Dickens et al., 2011). This time frame produced the most consistent increase in POM/BST AA for males and females. At the end of the restraint stress period, birds were removed from the metal cylinder and immediately sacrificed. Sample collection and processing continued as described for control birds.