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The effect of 17β estradiol withdrawal on the level of brain and peripheral neurosteroids in ovarectomized rats

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Abstract

Dehydroepiandrosterone (DHEA), pregnenolone (P) and their sulfate derivatives are neuroactive neurosteroids synthesized endogenously in the brain and in steroidogenic organs and influence or are influenced by a variety of physiological processes. Since parturition is followed by a rapid drop in estrogen levels in serum and brain it may be hypothesized that the drastic drop in the brain exposure to estrogens may cause a disturbance in the neurosteroid-to-neurosteroid-sulfate equilibrium with clinical relevance. In order to develop a rat animal model for human postpartum rapid estrogen decline conditions, the present study investigated effects of sudden withdrawal of hyperphysiological estrogens levels on levels of DHEA, DHEAS, P and PS in peripheral blood and brain tissue as well as cortical sulfatase activity. Twenty-four 3-month-old female rats were ovarectomized followed by either no estrogen, high levels of estrogen alone, or followed by sudden withdrawal after high-administered estrogen levels. Results indicated elevated brain cortical DHEA-S and reduced cortical sulfatase in ovarectomized rats following sudden estrogen withdrawal. No significant alterations in DHEA, P or PS were noted. Study observations suggest the marked influence estrogen withdrawal states may have on cortical DHEA-S levels in particular, the precise mechanism of which remains unknown but which may be related to the paralleled decrease in sulfatase activity. This DHEA-S increase may lead to attenuated GABAergic tone and may be relevant to post-natal behavioral disturbances (e.g. depression, anxiety).

Keywords: Postpartum; Neurosteroid; Dehydroepiandrosterone; DHEA; DHEA-S; Pregnenolone

Dehydroepiandrosterone (DHEA), pregnenolone (P) and their sulfate derivatives, dehydroepiandrosterone sulfate (DHEAS) and PS, are neuroactive neurosteroids that are synthesized endogenously in the brain and in steroidogenic organs [3]. Neuroactive steroids are naturally occurring steroids whose function includes that of the rapid alteration of the excitability of neurons through interaction of gamma-aminobutyric acid (GABA) gated ion channels. These receptor-active neurosteroids may represent an important class of neuromodulators, which act via novel non-genomic mechanisms. DHEA may have a direct or indirect $GABA_A$ agonistic-like activity [2,11,20], whereas the sulfated steroids are allosteric $GABA_A$ antagonists [1,10,14–16,22]. Both free and sulfated steroids are interconvertible via sulfatase and sulfotransferase activity [12]. The level of these "inhibitory" and "excitatory" neurosteroids, and the ratio between them, may be factors in the modulation of mood disorders such as anxiety and depression.

One such physiological condition, potentially affected by these changes, is parturition. Parturition is followed by a rapid drop in estrogen and progesterone levels in serum and brain [18]. Since following childbirth many women suffer from minor mood disturbances, depression as well as the more severe but less common postpartum psychosis, it may be hypothesized that the drastic drop in the brain exposure to estrogens may cause a disturbance in the neurosteroid-to-neurosteroid-sulfate equilibrium and thus may play a role in

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these postpartum depressive conditions. The present study investigated the effect of controlled exposure to hyperphysiological levels of estrogens followed by sudden withdrawal (in peripheral blood and cortical tissue homogenates of rats) on levels and ratio of the neurosteroids DHEA, DHEAS, P and PS as well as sulfatase activity in brain tissue. Since P is synthesized both by the adrenal and the gonads, while DHEA is synthesized only by the gonads (due to lack of p450 17 hydroxylase) [7], rats were ovarectomized in order to control for endogenous ovarian synthesis. Thus, levels of DHEA and DHEAS may inform regarding brain synthesis and brain to periphery diffusion, while P and PS may inform regarding both peripheral and brain synthesis. This approach thus serves as an animal model for the human postpartum condition with its sudden drop in estradiol (E_2) levels.

Our study was conducted on Sprague–Dawley female rats, housed three to four per cage and maintained on a 12-h light:12-h dark cycle. The study was approved by the Committee for Experiments in Animals, Life Science Faculty, Tel Aviv University, Ramat Aviv, Israel. Three groups of ovariectomized rats were tested. Twenty-four 3-month-old (180–200 g) female rats were ovariectomized under diethylether anesthesia. Ten days following ovariectomy the rats were divided into the three groups. The subgroups of rats were injected SC for 5 days as follows:

Experimental group
Days 1–2: 300 ng estradiol (E_2)/200 μl sesame oil intraperitoneal (i.p.)
Day 3: 300 ng E_2/200 μl DMSO i.p.
Days 4–5: 200 μl sesame oil i.p.
(E_2 withdrawal group: a model for a rapid drop of E_2 concentration in the brain after exposure to high E_2 levels. DMSO was used on the third day of E_2 administration instead of sesame oil in order to ascertain a rapid distribution in the body).

Corresponding control group
Days 1–2: 200 μl vehicle (sesame oil) i.p.
Days 3–4: 300 ng E_2/200 μl sesame oil i.p.
Day 5: 300 ng E_2/200 μl DMSO i.p.
(E_2 group: rats received 3 days of E_2 treatment preceded by 2 days of sesame oil injection in order to maintain same schedule of five injections in all scenarios and to obtain high brain levels of E_2 at the end of the fifth day).

Ovarectomized group
Days 1–5: 200 μl sesame oil i.p. (ovx: serves as control group).

All animals were decapitated 24 h after the last injection. Serum and plasma were subsequently collected for hormone evaluation and leukocyte separation respectively. The brain was removed in preparation for separation of the cortex. All organs were maintained at −70 °C.

(a) *Serum*: After decapitation, peripheral blood was collected in tubes without anticoagulant. After 30 min at room temperature, blood samples were centrifuged at 600 × *g* for 10 min. Serum was separated and kept at −70 °C until use.

(b) *Cortex homogenate*: Cortex homogenate was prepared for testing neurosteroid levels. Following decapitation, brains were removed, and the cortex separated and immediately placed on ice. The cortex was weighed and homogenized in 0.1 M Tris buffer pH 7.6 (1:2 w/v) by teflon homogenizer followed by sonication for 30 s to release the sulfatase enzyme, as described above. The sonicate was centrifuged at 10,000 × *g* for 45 min at 4 °C. The supernatant was separated, the volume measured, and kept at −70 °C until use. A cortex weighing about 1 g yielded a final volume of approximately 1.5 ml after centrifugation (0.5 ml were removed for DHEA, 0.6 ml for P and PS and 0.05 ml for DHEAS estimations).

Protein concentration was tested by BCA-protein assay kit (Pierce Laboratories, Illinois, USA). Absorbance was read using an Elisa Reader at 550 nm.

Sulfatase activity was determined using 7-^{3}H-DHEAS as a substrate (21 Ci/mmol; NEN, Boston, MA, USA). ^{3}H-DHEAS in ethanol was evaporated to dryness and dissolved in 0.1 M Tris buffer, pH 7.6 to yield a final concentration of 0.36 μM in the assay mixture. One hundred microliters 7-^{3}H-DHEAS in buffer, 100 μl 0.1 M Tris buffer pH 7.6 and 200 μl cortex homogenate (0.5 g/ml) were incubated for 3–4 h at 37 °C. The reaction was stopped by adding 3.0 ml diethylether, vortexed twice for 30 s and centrifuged at 350 × *g* for 5 min for phase separation. The aqueous phase was frozen at −20 °C and the organic phase was decanted into a scintillation vial, evaporated till dryness, dissolved in 3 ml scintillation fluid and counted in β-scintillation counter. Sulfatase activity is expressed as pmol DHEA synthesized/mg protein/hour.

(a) *Dehydroepiandrosterone*: DHEA level was measured using DSL 9000 ACTIVE™ DHEA coated tubes radioimmunoassay (RIA) kit (Diagnostic Systems Laboratories, Webster, TX, USA). 0.5 ml serum or cortex homogenate were extracted twice with 5.0 ml diethylether, centrifuged at 350 × *g* for 5 min and kept for about 15 min at −70 °C to allow the aqueous phase to freeze. The etheric phase was decanted, evaporated till dryness, and dissolved in 120 μl of standard 0 of the RIA kit. One hundred microliters were taken for determination of DHEA level.

(b) *Dehydroepiandrosterone sulfate*: DHEAS levels were measured using Immunochemo™ double antibody RIA kit ICN (Biomedicals Inc. Costa Meso, CA, USA). Serum was diluted 1:5 and 1:10 with steroid diluent (supplied within the kit) and cortex homogenate was diluted 1:2 before testing.

(c) *Pregnenolone*: Pregnenolone was measured using a modification of the commercial ICN Pregnenolone tritum RIA kit (Biomedicals, Costa Mesa, CA, USA). 0.1 ml 1:10 ^{3}H-P from the RIA kit were added to either 0.6 ml serum or 0.6 ml cortex homogenate as an internal stan-