Analysis of neurosteroid levels in attention deficit hyperactivity disorder

Rael D. Strous1, Baruch Spivak2,3, Rony Yoran-Hegesh2,3, Rachel Maayan4, Elena Averbuch2, Moshe Kotler5, Roberto Mester2,3 and Abraham Weizman3,4,6

1 Beer Yaakov Mental Health Center, PO Box 1, Beer Yaakov 70350, Israel
2 Ness-Ziona Mental Health Center, PO Box 1, Ness-Ziona 74100, Israel
3 Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel
4 Laboratory of Biological Psychiatry, Feinstein Medical Research Center, Beilinson Campus
5 Ben Gurion University Faculty of Medicine, Beer Sheba, Petach Tikvah 49100, Israel
6 Research Unit, Geha Psychiatric Hospital, Petach Tikvah 49100, Israel

Abstract

Neurosteroids are important neuroactive substrates with demonstrated involvement in several neurophysiological and disease processes. Attention deficit hyperactivity disorder (ADHD) has been associated with dysregulation of the catecholaminergic and serotonergic systems, however its relationship to irregularities or changes in neurosteroid levels remains unknown. We examined the relationship between blood levels of dehydroepiandrosterone (DHEA), its principal precursor pregnenolone and its principal metabolite dehydroepiandrosterone sulphate (DHEAS) in 29 young male subjects aged 7–15 years with DSM-IV criteria of ADHD. Subjects were evaluated by a specially designed scale, following which patients were divided into two groups according to severity of symptomatology. Results indicated significant inverse correlations between clinical symptomatology and levels of DHEA and pregnenolone in the total group. These inverse correlations were particularly evident in the less severe group of subjects. Levels of DHEA and DHEAS were inversely correlated with the hyperactivity subscale. Furthermore, using median blood levels as a cut-off indicator, higher blood levels of DHEA and DHEAS were associated with fewer ADHD symptoms, in particular hyperactivity symptomatology. Our findings suggest a possible protective effect of various neurosteroids on the expression of ADHD symptomatology.

Received 4 September 2000; Reviewed 10 December 2000; Revised 12 March 2001; Accepted 20 March 2001

Key words: ADHD, dehydroepiandrosterone, dehydroepiandrosterone sulphate, GABA, neurosteroids.

Introduction

Neurosteroids are important neuroactive substrates with demonstrated involvement in several neurophysiological and disease processes. They have been shown to affect expression of mood, energy, aggression and general activity (Wolkowitz et al., 1999). More specifically, dehydroepiandrosterone (DHEA), a major circulating neurosteroid in humans, has been demonstrated to play several vital neurophysiological roles and be affected by various physiological processes. These include neurotrophic and neuronal excitability effects, circadian rhythms, sexual response, immunological and stress reactions, memory, ageing and sleep (reviewed in Baulieu and Robel, 1996; Herbert, 1998). It is considered a neurosteroid, as it is produced in the brain; as well as a neuroactive steroid, as it is produced in the adrenals and has an effect on the brain. DHEAS, its sulphated form, is believed to be the most abundant steroid found in the body (Baulieu and Robel, 1996; Wolf et al., 1997).

Attention deficit hyperactivity disorder (ADHD) has been associated with dysregulation of the catecholaminergic and serotonergic systems (Sagvolden and Sergeant, 1998), however its relationship to irregularities or changes in neurosteroid levels remains unknown. This becomes especially important considering neurosteroid effects on monoaminergic and GABAergic neurotransmitter systems (Gasior et al., 1999; Majewska, 1992; Zinder and Dar, 1999). We examined the relationship between blood levels of DHEA, its principal precursor pregnenolone, and its principal metabolite dehydroepiandrosterone sulphate (DHEAS) in young male individuals with ADHD.
Method

Study population

Twenty-nine boys with DSM-IV-based criteria of ADHD, combined type, participated in the study. Subjects were recruited from referrals to a specialist child and adolescent mental health outpatient clinic. The diagnosis required the consensus of two certified child and adolescent psychiatrists (R.Y.H. and E.A.). Furthermore, for participation in the study, a minimum score of 15 on the Abbreviated Conner’s Teacher Rating Scale (ACTRS) (Conners and Barkley, 1985) was required. Based on subject and family member interview and chart review, all subjects were without comorbidity of conduct disorder or any other psychiatric disorder (e.g. depression). Prior to study onset, physical health and normal intelligence were confirmed by routine examination. Subjects were medication free for at least 2 months prior to the study. All subjects and their parents consented to participate in the study and all parents provided written, informed consent to this nature.

Clinical assessments

ADHD symptomatology was assessed by an index based on the DSM-IV and specially designed for ADHD, the DSM-IV ADHD Scale (DAS). This scale has previously been described and utilized by our group (Spivak et al., 1999). In summary, the DAS is comprised of two subscales, each of which is scored independently: the Inattention Subscale and the Hyperactivity–Impulsivity Subscale. Each DSM-IV ADHD criterion is rated on a 4-point Likert scale with the following qualification: 0 = not present; 1 = sometimes; 2 = often; 3 = very often present. Maximum score possible for each scale is 27, with a maximum total score being 54. Subjects were categorized based on the total DAS findings as either low-severity ADHD (DAS \leq 31) or high-severity ADHD (DAS > 31). This cut-off limit was selected in order to ensure a score of at least 2 in two-thirds of the items, and at least 1 in the remaining third (Spivak et al., 1999).

Laboratory testing

Since neurosteroid levels are variable during the day, the time of the peripheral blood testing was standardized and obtained between 08:00 and 09:00 hours in all subjects. Subjects were instructed to abstain from unusual physical activity or stress for a period of 24 h prior to blood sampling. DHEA was tested with the DHEA-DSL 9000 Active™ DHEA-coated tube radioimmunoassay (RIA) kit (Diagnostic System Laboratories, Webster, TX, USA); sensitivity 0.7 nmol/l; cross-reactivity with DHEAS 0.88%. DHEAS was tested with the DHEAS-DSL-3500 Active™ DHEAS-coated tube RIA kit (Diagnostic System Laboratories); sensitivity 4.6 nmol/l. Pregnenolone was tested with [³H]pregnenolone RIA kit (ICN Biomedical, Inc., Costa Mesa, CA, USA); sensitivity 0.04 nmol/l; cross-reactivity with other steroids is less than 0.05%. Cortisol was measured by the TKCO1 Coat-A-Count kit (Diagnostic Products Corporation, Los Angeles, CA, USA); sensitivity 13.8 nmol/l. Hormone levels in all samples were measured simultaneously to avoid interassay variability. The intraassay variability values for cortisol, DHEA, DHEAS and pregnenolone were 3–4.8, 5.6–10.6, 6.3–9.4 and 7–15%, respectively according to the level both between and within runs.

Statistical analysis

Two-tailed unpaired Student’s t test with unequal variance and Pearson’s correlation test were applied to data obtained.

Results

The 29 young male subjects ranged in age from 7 to 15 yr with an average age of 10.6 yr (s.d. = 1 yr). In this total group of ADHD subjects, average neurosteroid blood levels obtained were: for DHEA 10.48 nmol/l (s.e.m. = 7.07, range = 1.2–26.4, no normal range is available according to the laboratory kit manual); for DHEAS 1409.17 nmol/l (s.e.m. = 1171.65, range = 176–4740, normal prepubertal range = 54–1165 nmol/l); for pregnenolone 7.25 nmol/l (s.e.m. = 3.07, range = 2.37–15.1, normal prepubertal range = 1.6–10.4 nmol/l); and for cortisol 231.13 nmol/l (s.e.m. = 95.47, range = 62–450, normal range = 28–1100 nmol/l). In the high-severity ADHD subgroup, average neurosteroid blood levels obtained were: for DHEA 8.74 nmol/l (s.e.m. = 6.76, range = 1.2–26.3); for DHEAS 1226.4 nmol/l (s.e.m. = 1222.64, range = 176–4666), for pregnenolone 6.84 nmol/l (s.e.m. = 3.29, range = 2.37–12.29), and for cortisol 230.4 (s.e.m. = 119.6, range = 62–450). Finally, in the low-severity ADHD subgroup, average neurosteroid blood levels obtained were: for DHEA 12.35 nmol/l (s.e.m. = 7.43, range = 2.2–26.4); for DHEAS 1605 nmol/l (s.e.m. = 1171.59, range = 237–4666), for pregnenolone 7.76 nmol/l (s.e.m. = 2.88, range = 4.74–15.1), and for cortisol 231.8 (s.e.m. = 70, range = 94–324).

See Figure 1 for representation of levels of DHEA, DHEAS, pregnenolone, cortisol and DHEA/cortisol in both low- and high-severity ADHD subgroups. In addition, DHEA/cortisol ratios were computed since the
ratio has been reported to be useful in accounting for variance of DHEA levels when present. Results indicated significant inverse correlations between clinical symptomatology (as assessed by DAS) and blood levels of DHEA \((n = 29, r = -0.44, p < 0.05)\) and pregnenolone \((n = 29, r = -0.38, p < 0.05)\) in the total group. These inverse correlations were mostly as a result of levels of DHEA and pregnenolone in the low-severity \((DAS \leq 31)\) ADHD subgroup \((DHEA: n = 14, r = -0.57, p < 0.05;\) pregnenolone: \(n = 14, r = -0.56, p < 0.05)\), since correlation of levels in the high-severity subgroup did not reach significance. DHEA/cortisol ratios, however, showed an inverse correlation with symptomatology in only the high-severity subgroup \((n = 14, r = -0.56, p < 0.05)\). In the total group, ratings on the hyperactivity subscale of the assessments were inversely correlated with levels of DHEA \((n = 29, r = -0.45, p < 0.05)\) and DHEAS \((n = 29, r = -0.38, p < 0.05)\), respectively. There were no correlations between age of subjects and DHEA, DHEAS or pregnenolone levels in our ADHD patients.

Having noted these observations, we divided DHEA levels obtained from the total subject group into two subgroups using the median level of 8.4 nmol/l. A comparison of DSM-IV symptomatology (total DAS score) between the two DHEA subgroups indicated less ADHD severity in the subgroup with the higher DHEA blood levels \((t = -2.35, d.f. = 26, p < 0.05)\). In particular, subjects with higher DHEA levels, had fewer hyperactivity symptoms \((t = -2.34, d.f. = 26, p < 0.05)\). This effect of higher DHEA levels being associated with less severity of ADHD symptoms was not noted on the impulsivity symptom subscale. In a similar fashion, DHEAS levels were divided into two subgroups using the median of 1167 nmol/l. Once again, comparison of DSM-IV symptomatology severity between the two DHEAS

**Figure 1.** Levels of neurosteroids in high- and low-severity ADHD subgroups.
subgroups indicated less severity in the group with the higher DHEAS blood levels ($t = -2.98$, df. = 26, $p < 0.01$), with the more pronounced effect being on hyperactivity ($t = -2.93$, df. = 27, $p < 0.01$) and not impulsivity symptoms.

In all three groups (total sample, high severity, low severity) levels of DHEA and DHEAS correlated highly with each other ($n = 29, r = 0.82$; $n = 15, r = 0.97$; $n = 14, r = 0.66$; $p < 0.01$ for all). Similarly, levels of DHEA and pregnenolone correlated highly with each other ($n = 29, r = 0.73$; $n = 15, r = 0.61$; $n = 14, r = 0.87$; $p < 0.01$ for all). No correlations with DHEA or pregnenolone on the ACTRS was noted. No differences were noted on $t$ test analyses of DHEA, DHEAS and pregnenolone levels between low-severity and high-severity groups. However, significant differences were noted between the DHEA/cortisol ratios in these two severity groups ($p = 0.05$).

Conclusions

Results from this study indicate inverse correlations between clinical symptomatology and levels of DHEA and pregnenolone, particularly evident in patients with a lesser severity of ADHD. In addition, higher blood levels of DHEA and DHEAS were associated with fewer ADHD symptoms, in particular hyperactivity symptomatology. While our findings may suggest a possible protective effect of DHEA and DHEAS on the expression of ADHD symptomatology, correlation does not establish causation and the described relationship may be an indirect epiphenomenon. The lack of a control group further interferes with any firm assumption along these lines. Nevertheless while speculative, it may still be hypothesized that a certain threshold of DHEA and DHEAS blood levels is required in order to attenuate the severity of ADHD symptoms and thus to engage a possible, yet unproven, neurosteroid protective effect. If this threshold of DHEA and DHEAS levels is not attained, the protective effect may be lost, and will result in enhanced ADHD symptomatology observed.

DHEA activity in the brain is thought to involve several neurophysiological pathways, including stimulatory or antagonistic effects at GABA$_A$ receptors, facilitation of NMDA and sigma neurotransmission, the increase of serotonin levels in the brain as well as the antagonistic action against certain cortisol effects (Wolkowitz et al., 1999). While it remains unclear which of these effects may play any particular role in ADHD patients, it has been hypothesized that the ‘calming’ effects of the neurosteroids may be achieved via direct or indirect effects on GABA-mediated chloride ion conductance (Majewska, 1995; Robel and Baulieu, 1995; Zinder and Dar, 1999). This is despite DHEA and DHEAS’s demonstrated GABA$_A$ allosteric antagonistic properties (Majewska, 1992; Spivak, 1994). Thus, they may act by either significantly decreasing levels of the GABA$_A$ antagonist-like pregnenolone sulphate (Robel and Baulieu, 1995) or by increasing levels of GABA$_A$ agonist-like progesterone metabolites (Young et al., 1998) or androsterone, a DHEA metabolite (Majewska, 1995). Others, however, have suggested that DHEA mediates its anxiolytic effect via mechanisms independent of GABA$_A$ receptor-mediated chloride uptake (Imamura and Prasad, 1998). It should also be noted that pregnenolone, the predominant precursor of DHEA as described above, has also been shown to mediate an anxiolytic effect by virtue of its action at the GABA$_A$ receptor (Bitran et al., 1999). Moreover, following these observations, DHEA has been demonstrated in several animal studies to decrease some forms of aggression (Baulieu and Robel, 1996; Herbert, 1998). This is despite at least one study in which an association of higher DHEAS levels with aggression in conduct disorder has been observed (van Goozen et al., 1998). The precise mechanism of these described effects is not completely understood, but it has been suggested that DHEA may lead to an increase in GABAergic tone via indirect neurotransmission potentiation (Baulieu and Robel, 1996). Interestingly, considering the efficacy of methylphenidate in the management of ADHD and based on the knowledge that striatal dopaminergic neurotransmission appears to be under the regulatory control of GABAergic inputs, Tirelli et al. (1998) demonstrated augmentation of methylphenidate activity by means of GABA$_A$ agonist activity, a potential indirect or secondary effect of DHEA as described above. While these above indirect mechanisms of DHEA action at the GABA$_A$ receptor certainly remain possible, alternatively it still may be feasible that DHEA’s more direct antagonistic activity at the receptor plays a role in the diminution of ADHD symptomatology.

A further hypothesized mechanism of DHEA efficacy in the ADHD population may be that of NMDA or glutamate-enhanced neurotransmission via sigma receptors (Bergeron et al., 1996; Monnet et al., 1995). While no definitive studies of NMDA or glutamate activity appear to have been completed in ADHD patients, Oranje et al. (2000) demonstrated attention deficits and impairment with the NMDA antagonist ketamine. Furthermore, Tang et al. (1999) demonstrated in mice that genetic enhancement of signal detection by NMDA receptors assists in learning and memory. Thus DHEA may exert positive effects in ADHD patients by means of improved neurotransmission at these receptors.

In humans, DHEA has been shown to increase the sense of well-being, energy, stamina and libido in middle-aged
and elderly individuals (Kalmijn et al., 1998; Wolf et al., 1997), as well as in patients with multiple sclerosis and systemic lupus erythematosus (Barry et al., 1998). Interestingly, in a recent double-blind placebo-controlled study in patients with Alzheimer’s disease even cognitive scales were shown to improve with DHEA administration (Wolkowitz et al., 2000). In addition, another double-blind study has replicated previous anecdotal reports of the efficacy of DHEA in the management of depression (Wolkowitz et al., 1999). These encouraging effects on mood may be hypothesized to take place via effects on monoaminergic receptor systems, similar positive effects of which are observed with various medication treatment schedules in ADHD, e.g. methylphenidate (Solanto, 1998).

Since DHEA administration has been shown to have a mood-elevating and even antidepressant effect in certain populations as described above, based on our preliminary findings it may be hypothesized that positive results on behaviour with DHEA administration may be expected in the ADHD population. Several recent studies have suggested the important role that neuroactive steroids may have in future clinical practice (Gasiore et al., 1999; Wolkowitz et al., 1999). Whether these positive results may extend to the ADHD population, despite their expected neuroendocrine side-effect profile, remains unknown. Further studies of a double-blind nature are clearly warranted in order to test these observations and assumptions.

References


in conduct disorder prepubertal boys and normal controls. Biological Psychiatry 43, 156–158.