Age-Related Differences in the Regulation of Dehydroepiandrosterone Sulfate Levels in Peripheral Blood Plasma of Monkeys

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Although dehydroepiandrosterone (DHA) and its sulfate (DHAS) are quantitatively the major corticosteroids present in the human circulation, their physiological significance and regulation are not yet fully understood. There is evidence in the literature suggesting that these compounds may play important roles in the regulation of lipid and carbohydrate metabolism [5,7,12] and of immunological status [15] and also in the pathogenesis of atherosclerosis and malignant neoplasms [8,12,14]. Most of the relevant experimental studies have used small laboratory animals which differ substantially from man in patterns of DHAS secretion. It is therefore of considerable interest to examine how DHAS secretion is regulated in certain simian species, in particular sacred baboons, which have been shown [1] to be an appropriate animal model for studying various aspects of steroidogenesis and of the secretion and metabolism of steroid hormones.

In this study, the regulation of DHAS levels in the circulation of young sexually mature and old infertile male sacred baboons was investigated using a set of functional tests including those with ACTH, insulin, dexamethasone, human chorionic gonadotropin (HCG), luliberin (luteinizing hormone-releasing hormone - LH-RH), the D-Ser(Bu^t)^6-ethylamide of the latter, and a highly sensitive and specific direct radioimmunoassay for plasma DHAS specially developed for such studies.

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MATERIALS AND METHODS

Twenty young sexually mature (aged 8-10 years) and 2 old (aged 26 years) infertile male sacred baboons kept in the Sukhumi and Adler Primatological Centers were used. Before the experiment, the animals were adapted to the experimental conditions (maintenance in individual metabolic cells and the blood-sampling procedure).

Blood samples were taken in intact males and in those whose adrenal function was stimulated (by ACTH or insulin) or inhibited (by dexamethasone) as well in those whose gonadal function was activated by HCG or LH-RH or inhibited through prolonged infusion of an LH-RH agonist.

TABLE 1. Diurnal Rhythms of DHAS and F Levels in the Peripheral Blood Plasma of Male Sacred Baboons Aged 8–10 Years ($n=5$). The Values are Means±SEM

Time of day, h	Concentration, nmol/liter	
	DHAS	F
9:00	190±26	1030±130
15:00	110±40	640±88*
21:00	120±20*	690±70*
3:00	160±16	890±50
9:00	206±27	1230±70

Note. Asterisk marks a significant difference at $p<0.05$.

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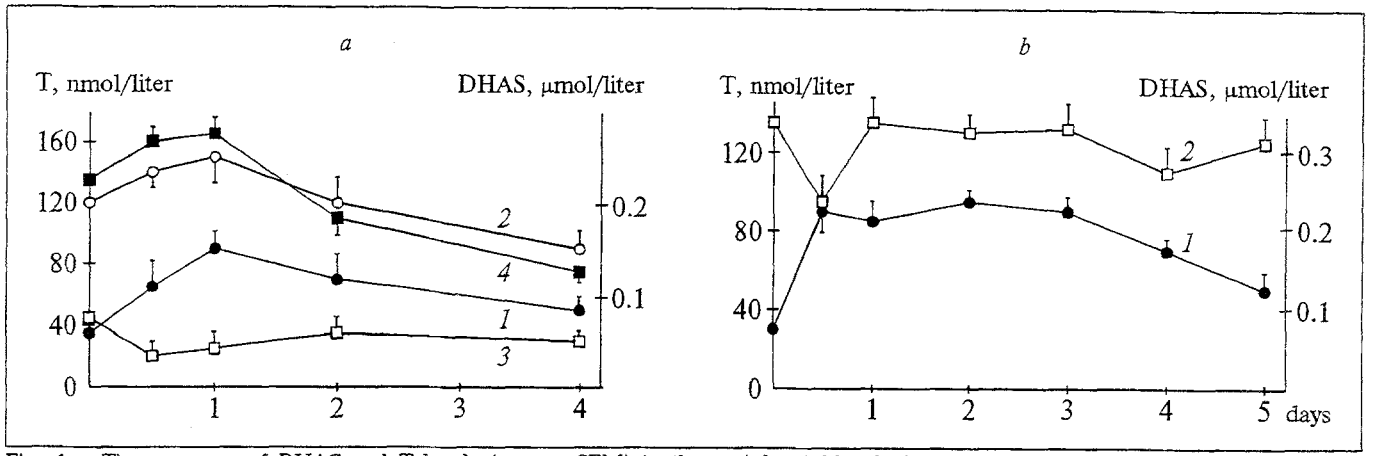

Fig. 1. Time courses of DHAS and T levels (means±SEM) in the peripheral blood plasma of young sacred baboons in response to LH–RH (*a*) and HCG (*b*) administration. *1*) T after LH–RH or HCG; *2*) DHAS after LH–RH or HCG; *3*) T after physiological saline; *4*) DHAS after physiological saline.

ACTH (suspension of zinc corticotropin from the Kaunas Plant of Endocrine Preparations) was administered intramuscularly, 1 U/kg body weight; insulin (Actrapid; Novo Ind., Denmark) intravenously, 0.2 U/kg; dexamethasone (Galenika, Yugoslavia) intramuscularly, 8 mg/day for 2 days; HCG (Gonadex; LEO, Sweden) intramuscularly, 1500 U/kg both to intact animals and those in whom gonadal function was inhibited. The LH-RH agonist (D-Ser(Bu^t)^6-ethylamide of LH-RH or Buzerelin; Hoechst A.G., Germany) was infused in a dose of 3 or 12 μg/kg per day for 11-16 weeks by means of osmotic minipumps (Alza Corp., Palo Alto, USA) and LH-RH (Lutrelef; Ferring, Germany) was injected intravenously, 100 μg per animal. The compounds were administered between 10:00 and 11:00 h. Blood was sampled before their administration and 15, 30, 60, 120, 240 min and 24 h thereafter, and additionally, for a study of the diurnal rhythms of DHAS secretion, at 9:00, 15:00, 21:00, 3:00, and 9:00 h on the following day. It was taken from the ulnar vein using heparin as anticoagulant. Plasma was separated by centrifugation and stored at -20°C prior to analysis.

Testosterone (T) was determined by radioimmunoassay (using a RIN-^{3}H-T reagent kit [2]), and cortisol(F), by a competitive binding assay [13]. Plasma DHAS levels were estimated by a specific and sensitive method of direct radioimmunoassay described in detail previously for use with human plasma [3]. For the assay of DHAS in baboon plasma, 1-2 μl of native plasma were used. The sensitivity of the method was 0.039 μmol/liter. The reproducibility of the results did not exceed 10% within assays and 15% between series of assays. This method met the test requirements for parallelism in determining DHAS in plasmas of both young and old animals in the 0.5-8 μl range.

Correlation analysis and Student's *t* test were used for statistical treatment of the results.

RESULTS

DHAS concentrations in the peripheral blood plasma of young mature sacred baboons ranged from 60 to 760 nmol/liter, the mean value being 270±16 nmol/liter (the 95% confidence interval, 240-300 nmol/liter). In the old baboons, DHAS levels were significantly lower ($p<0.001$) - 35±2 nmol/liter (range 21-50 nmol/liter).

The diurnal rhythm of DHAS levels in the circulation of young baboons correlated closely ($r=0.976$) with the circadian variation in F levels (Table 1). Acute stress (insulin hypoglycemia) or ACTH administration activated F secretion as well as inducing an elevation of the DHAS level, whose time course also correlated closely with that of F ($r=0.860$ for the acutely stressed animals and $r=0.971$ for those injected with ACTH). Dexamethasone injections exerted similar inhibitory effects on DHAS and F levels (Table 2). These findings indicate that in young baboons DHAS is predomi-

TABLE 2. Time Course of DHAS and F Levels in the Peripheral Blood Plasma in Male Sacred Baboons aged 8–10 Years ($n=3$) in Response to Dexamethasone Injected Intramuscularly at 2 mg Four Times Daily for Two Days

Day after dexamethasone	Concentration, nmol/liter	
	DHAS	F
0	98±19	1140±80
1st	62±16	430±98**
2nd	26±7*	292±55**

Note. Asterisks indicate a significant difference at $p<0.01$ (*) and $p<0.001$ (**).

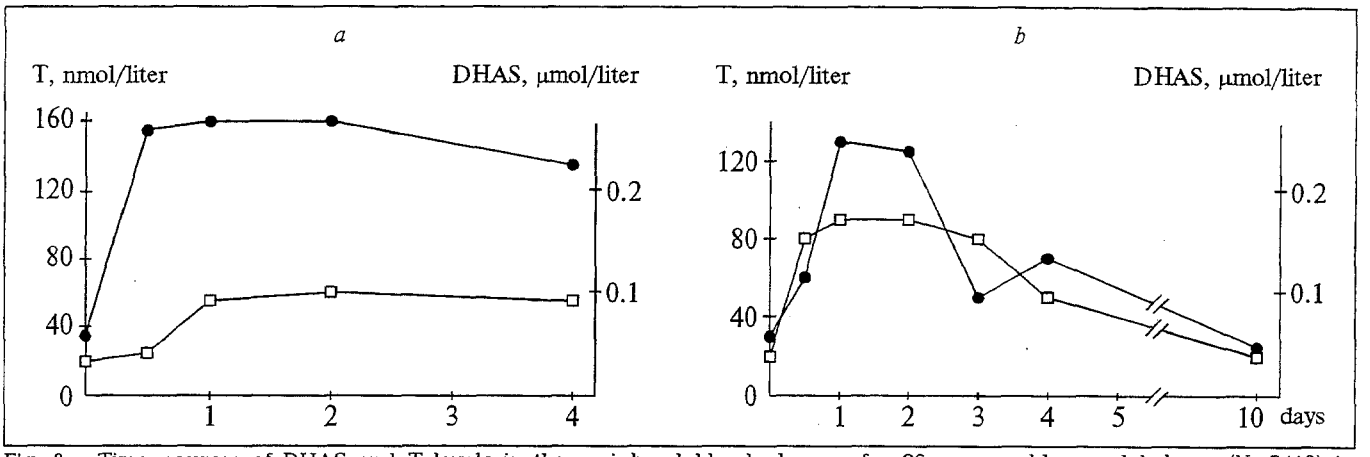

Fig. 2. Time courses of DHAS and T levels in the peripheral blood plasma of a 26-year-old sacred baboon (№ 7419) in response to LH-RH (*a*) and HCG (*b*) administration. *1*) T; *2*) DHAS.

nantly secreted by the adrenals and that a leading role in regulating the secretion of adrenal hormones is played by ACTH.

Although circulating F and DHAS levels of young baboons correlated closely under conditions of stimulated as well as inhibited adrenocortical function, some differences were noted in the time-course and degree of their variations. Thus, elevations in F levels in response to the acute stress and ACTH administration were greater and lasted longer than did those in DHAS levels. The response of F to dexamethasone was also more strongly marked. This suggests that F is more dependent than DHAS on ACTH. In baboons, as in man, certain other factors in addition to ACTH appear to participate in the regulation of DHAS secretion.

The reported evidence for the possibility of DHAS secretion by human gonads [4,10] and those of rhesus monkeys [9] suggests that gonads could

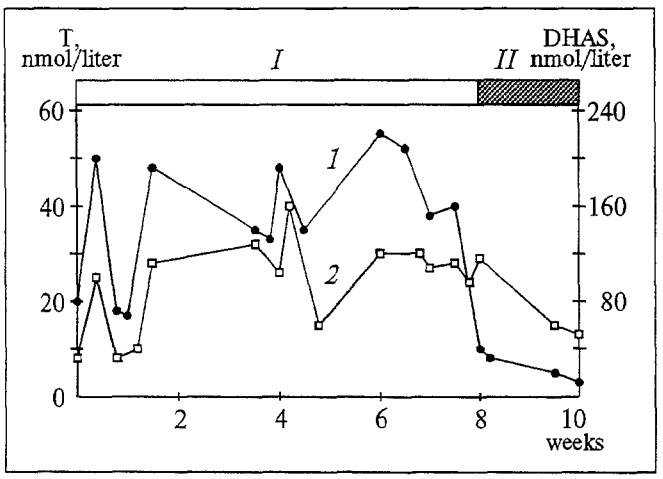

Fig. 3. Time courses of T (*1*) and DHAS (*2*) in the peripheral blood plasma of the same baboon as in Fig. 2 (№ 7419) after a prolonged (for 10 weeks) tonic administration of Buzerelin in 3 μg/kg per day during the first 8 weeks (*I*) and at 12 μg/kg per day during the last 2 weeks (*II*).

contribute to the formation of a DHAS pool in the peripheral blood of baboons and that, accordingly, gonadotropins and LH-RH might play an important role in the regulation of DHAS secretion. However, no reductions in DHAS were found to have occurred in the young baboons whose gonadal function was inhibited through prolonged infusion of the LH-RH agonist Buzerelin. Thus, 12 weeks after its administration, the mean plasma level of DHAS was 353±50 nmol/liter and that of T less than 3 nmol/liter vs. 299±29 and 32±2 nmol/liter, respectively, before its administration (n=8). These findings permit the conclusion that the gonads of young sacred baboons do not contribute appreciably to the DHAS pool in the peripheral blood.

This conclusion is supported by measurements of plasma DHAS after the stimulation of testicular steroidogenesis with LH-RH (Fig. 1, *a*) or HCG (Fig. 1, *b*) in intact baboons or with HCG in those subjected to prolonged Buzerelin treatment to inhibit gonadal function (Table 3). In neither case did the pattern of variations in DHAS differ from that in baboons administered physiological saline.

While a single HCG injection failed to alter plasma DHAS levels significantly in most baboons, one baboon with Buzerelin-induced inhibition of gonadal function showed steep (2-3-fold) rises of DHAS after the HCG injection (Table 3). Rises of this hormone also occurred in most animals during the first 3 days of Buzerelin infusion in the presence of highly activated testicular steroidogenesis. Thus, the mean DHAS level was 290±50 nmol/liter 24 h after the start of Buzerelin administration and 420±80 nmol/liter at 48 h, as compared to the baseline level of 150±30 nmol/liter (n=9). This should be taken as evidence that the gonads of young baboons are capable of secreting DHAS in extraordinary situations attended by their hyperstimulation.

TABLE 3. Time Course of DHAS and T Levels in Response to HCG (1500 U) in the Peripheral Blood Plasma of Sacred Baboons Aged 8–10 Years Subjected to Prolonged (for Five Weeks) Infusion of Buzerelin or Physiological Saline

Time after HCG, days	Concentration, nmol/liter			
	DHAS	T	DHAS	T
	n=4		n=1	
	BUZERELIN			
0	385±68	2.8±0.1	235	3.9
0.08	290±46	59±12***	339	139
0.25	225±60*	92±13***	488	132
1	460±80	94±10***	753	125
2	410±65	94±13***	528	139
3	350±80	109±8***	615	125
	SALINE (n=5)			
0	285±30	2.8±0.1		
0.08	213±27*	2.7±0.3		
0.25	159±13**	2.4±0.4		
1	300±32	2.9±0.3		
2	294±43	3.4±0.3		
3	277±54	2.8±0.3		

Note. Asterisks indicate a significant difference at $p<0.05$ (*), $p<0.01$ (**), and $p<0.001$ (***).

The old baboons, unlike the young ones, responded to single injections of LH-RH (Fig. 2, *a*) or HCG (Fig. 2, *b*) with a marked elevation of DHAS levels, whose time course correlated closely with that of T levels, pointing to a testicular origin of DHAS; moreover, DHAS secretion by the gonads appears to have exceeded that by the adrenals since the gonadotropin-induced DHAS levels were several times higher than the baseline levels.

Stimulation of DHAS secretion was also observed in the old baboons during their prolonged exposure to Buzerelin in a dose insufficient to inhibit the pituitary-gonad system (Fig. 3). The subsequent suppression of testicular steroidogenesis by a higher Buzerelin dose reduced DHAS to nearly baseline levels.

The present study has shown that the main factor regulating the DHAS level in the circulation of young sexually mature baboons is ACTH. With aging, however, DHAS levels decline drastically, and LH-RH and gonadotropins become important for the regulation of DHAS secretion. Testicular secretion of DHAS can also occur in young animals when their gonadal function is hyperactivated. Monkeys may possess a reserve mechanism which can activate testicular steroidogenesis (by stimulating synthesis of sulfate-conjugated precursors and their transformation into T) and which plays a major role in androgenopoiesis in old animals. Possibly, it is through the operation of this mechanism that T concentrations characteristic of young animals were maintained in the old males for a relatively long time during their continued treatment with the LH-RH agonist in a dose too low to inhibit the pituitary-gonad system (Fig. 3).

A similar pathway for activation of testicular steroidogenesis may exist in human beings, given that elevations of DHAS along with other sulfate-conjugated precursors in the chain of T biosynthesis have been observed in men subjected to numerous HCG injections [10], and that *in vitro* experiments have demonstrated a direct stimulatory effect of HCG on the synthesis and secretion of sulfuric acid-conjugated T precursors by testicular tissue from elderly individuals [4]. The existence of such a pathway would improve the adaptive capability for androgenopoiesis in the aging organism, in which testicular steroidogenesis and its regulation are compromised [6,11].

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PHARMACOLOGY

GABA-Ergic Receptor System of the Myometrium: a Basis for a Clinical Study of GABA-Positive Substances as Gravidoprotectors

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γ-Aminobutyric acid (GABA) is known to take part in reproductive function regulation by controlling gonadotropin secretion by the hypothalamo-pituitary system [2,6,7]. GABA itself [5,9], its receptors [1,3,9], and also the benzodiazepine binding sites [4,8] have been identified in rat and rabbit ovaries and uterus.

The aim of this study was to investigate the function of the uterine GABA- and benzodiazepine (BD) receptors and to determine the influence of GABA-ergic substances on uterine contractility.

MATERIALS AND METHODS

Three series of experiments were performed. In the first series (35 experiments) the radioligand method was used to study the affinity of GABA and BD receptors of the nonpregnant uterus. We used a histologically normal myometrium obtained after uterine myoma resection. ^{14}C-GABA and ^{3}H-flunitrazepam (BD receptor ligand) were used for analysis. The results were processed by the Scatchard method. In the experiments of the second series (69 rats) radioligands were used to study the affinity of nonpregnant rats in the diestrus phase, of rats in the first half of pregnancy (10 days), and of rats in the second half of pregnancy (10-20 days). In the third series a pharmacological analysis of the GABA-BD receptor systems was performed. The experiments were carried out on the basis of the spontaneous contractility model on isolated fragments of uterine horns. We used 12 ovariectomized rabbits, 8 nonpregnant rabbits, and 12 rats (40 experiments). The $GABA_A$ agonists muscimol and ethylenediamine, the $GABA_A$ an-

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