REGULATORY MODIFICATIONS OF STEROIDOGENESIS FOLLOWING THE ADMINISTRATION OF EMBRYONARY PEPTIDES

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Regulatory modifications of steroidogenesis were induced following the administration on rats and humans of a nutritive supplement, Humanofort™, containing two embryonary peptides (EP) of 5 and 10 kDa. The administration of EP to rats for 60 days increased significantly the urinary excretion of 17-ketosteroids in all animals. The serum cholesterol increased only in females, by ~50%, this increase being associated with triggering the oestral cycle. The administration of EP to 30 young sportsmen (rugby players) for three weeks increased significantly serum androgen hormones (dehydroepiandrosterone, testosterone, androstenedione). The administration of EP to 40 old subjects, between 50 and 75 years of age (22 women and 18 men), induced adaptive-regulatory modifications of several steroids (cholesterol, testosterone, cortisol) towards physiological limits. In all subjects, the initial values of total cholesterol and LDL fraction exceeded the physiological range. After the administration of EP, these compounds decreased by ~30% in all subjects. Urinary 17-ketosteroids and some serum hormones (dehydroepiandrosterone, cortisol, testosterone) were significantly modified in ~70% of the subjects. At the end of the study, the levels of these hormones were within physiological range. The adaptive-regulation of steroidogenesis following the administration of EP depends on subjects' age, gender and initial biochemical status. An involvement of hypothalamus-pituitary-adrenal axis is strongly credited.

Key words: lipids, steroidogenesis, embryonary peptides.

INTRODUCTION

It is known that growth factors, mostly peptides with low molecular weight, act as chemical signals by triggering adaptive-regulatory modifications of essential processes in organisms (1–3). Among the members of growth factors family, still incomplete, the most known are the insulin-like growth factor, epidermal growth factor, nerve growth factor and fibroblast growth factor. These peptides easily penetrate cell membranes, acting upon specific receptors by triggering neuroendocrine reactions (3, 4).

Humanofort™ is a purified, standardized extract of embryonary peptides (EP), used as nutritive supplement. Preliminary studies showed that EP consisted of two peptides with molecular weight of 5 and 10 kDa and it does not contain prions or viral particles. The administration of EP is without toxicity or mutagenicity. A clinical study on elders showed that EP produced a significant decrease of the oxidative stress, triglycerides and cholesterol in the blood (5). In this paper we present the effects of EP administration in both animals and humans (young and old), related to a potential regulation of steroidogenesis.

MATERIAL AND METHODS

Humanofort™, a nutritive supplement, is a purified extract from embryonated chicken eggs, prepared by a procedure licensed OSIM No. 112, 102 (1997). Each cap contains 50 mg EP and excipient. Humanofort (EP) received the approval of the Ministry of Health of Romania (No. 5675/11.11.2002) and of the Food and Drugs Administration, USA.

The experimental study was performed at the Institute of Hygiene from Bucharest on 40 rats (20 males of weight 97 ± 4 g and 20 females of 95 ± 5 g). The rats were fed with a standardized diet. The EP extract was administered orally as 50 mg/kg body/day (as active compounds), for 60 days. No side effects were reported. Finally, all animals were sacrificed and blood was collected. Morning (basal) urine was collected directly from the bladder.

The young subjects were 30 sportsmen (rugby players), 20 to 30 years old, participating in this study as volunteers. They were divided into 3 group of 10 individuals. The first lot (control) received only Placebo caps . The second received 4 caps/day of EP and the third, 12 caps/day. The administration of EP lasted 21 days. Before and after this term, blood was collected from all subjects. For each individual, the initial and final biochemical markers were determined.

The old subjects were volunteers recommended by their family physician. The subjects (22 women and 18 men), between 50 and 75 years of age, were in satisfactory clinical health. Those with some medical problems were in chronic state, under medical control. No diet restriction was imposed. The subjects had taken 4 caps/day of EP (two in the morning and two in the evening), for 60 days. Before and after treatment, blood and urine from 24 hours were collected. Therefore, each subject was his own control. No side effects or allergic reactions were reported during trial.

Biochemical assays. The biochemical determinations were performed by Synevo Medicover Lab. (ISO-UE international accredit). The biochemical assays

were performed according to the methods recommended for UE and the results were processed by computers and presented as arithmetic mean ± standard error.

RESULTS AND DISCUSSION

EXPERIMENTAL STUDIES

Sixty days after the administration of EP to the two lots of rats (males and females), all animals were sacrificed and biological samples were collected. The results of biochemical assessments are presented in Table 1.

Table I

Biochemical assays performed on rats during the experimental study

Parameter	Control		EP administered	
	Males	Females	Males	Females
17-Ketosteroids (mg/g creatinine)	0.11 ± 0.02	0.16 ± 0.05	0.27 ± 0.07 ($p < 0.01$)	0.54 ± 0.04 ($p < 0.001$)
Cholesterol (mg/dl)	116.7 ± 5.3	88.2 ± 6.4	111.6 ± 7.2 (ns)	131.5 ± 9.2 ($p < 0.05$)
Total lipids (mg/dl)	226.4 ± 12.8	189.3 ± 16.2	245.2 ± 9.6 (ns)	255.1 ± 12.7 ($p < 0.05$)

No significant differences were observed in the body weight, nor in the level of hematological parameters, regardless of gender. However, the biochemical parameters showed significant differences, depending on the sex of the animals. The administration of EP induced a high significant increase of 17-ketosteroids in urine. A significant increase of serum cholesterol and lipids was noticed in females only. Our results showed that only in females the administration of EP produced a significant increase of steroidogenesis associated with lipidogenesis.

CLINICAL TRIAL ON YOUNG SPORTSMEN

The aim of the clinical trial on young subjects was to test the possible effect of EP on anabolic steroids, which may strongly influence the muscular mass, an essential factor for performances in sports. We used various concentrations of EP to test a possible dose-effect relationship. The results are presented in Table 2. One can observe that an important increase of the level of androgen hormones occured following the administration of EP. Due to the great individual differences, the final results were significant only when compared to the initial values. No significant result was observed for dehydroepiandrosterone, which is an important intermediate of androgen steroidogenesis, while significant increases of androstenedione and testosterone

Table 2

The effect of EP administration on some serum steroids in sportsmen

Hormone	Treatment		
	Placebo	6 caps EP/day	12 caps EP/day
DHEA	121.3 ± 16.5	89.7 ± 10.4	115.2 ± 13.8
DHEA sulfate	8l.4 ± 14.2	114.7 ± 11.5	108.3 ± 10.6
Androstenedione	88.5 ± 12.4	137.3 ± 21.5	126.5 ± 16.4
Testosterone	102.4 ± 14.7	124.5 ± 15.6	135.3 ± 17.9

Note: The results are presented as percentages relative to the initial values.

were noticed. A dose-effect relationship was observed only for testosterone, which is the principal androgen hormone. It is known that androgen hormones increase the muscular mass of the body, a requirement for performance sportsmen (1, 2).

CLINICAL TRIAL ON OLD SUBJECTS

We obtained more clear-cut results in the clinical trial that involved subjects aging between 50 and 75 years. They received a unique dose of EP, for a longer period of time. The obtained results are presented in Table 3. One can observe that in old subjects

Table 3

The effect of EP administration on some parameters in elder subjects

Parameter	Men		Women	
	Initial	After EP treatment	Initial	After EP treatment
Total cholesterol (mg/dl)	248.4 ± 6.3	216.6 ± 9.4 ($p < 0.01$)	274.6 ± 4.3	234.5 ± 9.2 ($p < 0.01$)
LDL-cholesterol (mg/dl)	156.5 ± 5.3	117.6 ± 8.5 ($p < 0.01$)	174.8 ± 4.3	140.6 ± 7.2 ($p < 0.01$)
17-Ketosteroids (mg/24h)	27.9 ± 4.8	21.4 (65%) 32.5 (35%)	23.7 ± 5.4	21.6 (52%) 27.2 (48%)
Cortisol (nmol/l)	486.5 ± 24.2	373.6 ± 28.7 ($p < 0.01$)	475.8 ± 24.3.	418.5 (72%) 496.7 (28%)
DHEA sulfate (μg/dl)	140.6 ± 21.8	108.5 (67%) 170.7 (33%)	81.8 ± 12.4	67.9 (76%) 121.6 (24%)
Testosterone (nmol/l)	14.7 ± 8.4	11.6 (72 %) 17.5 (28%)	–	–
IGF-1 (ng/dl)	77.4 ± 8.3	54.7 (77%) 83.6 (23%)	80.6 ± 9.4	62.5 (75%) 88.7 (25%)

DHEA = dehydroepiandrosterone; IGF = insulin-like growth factor. For some hormones, EP administration resulted in either an increased or a decreased value.

the initial values of cholesterol and its LDL-fraction were increased as compared to physiological range (150–210 mg/dl). The administration of EP induced in all subjects a significant decrease (mean of 30 mg/dl), regardless of gender. As the initial values of HDL-cholesterol were within physiological range, no important modifications were noticed. It is known that the synthesis and regulation of steroid hormones are complex processes (1) and thus the administration of EP produced diverse modifications, mostly regulatory changes of different intermediates, as function of sex and initial values.

For example, cortisol was initially increased in the majority of subjects, as a consequence of aging (6). Following the administration of EP, cortisol returned to physiological range in 70% of women and 100% of men. In most cases, the decrease of cortisol reached more than 50 nmol/l and most of regulative modifications of steroid hormones amounted for 70% of the subjects.

It should be also mentioned the significant decrease of growth factor IGF-1 in almost 70% of subjects; finally it returned to physiological limits.

Our results obtained by experimental and clinical studies demonstrate the adaptive-regulatory effects on steroidogenesis following a long-term administration of EP. As observed in Table 1, the administration of EP to rats induced a significant excretion of 17-ketosteroids in both sexes. It is known that these steroid metabolites are mostly synthesized in adrenal gland (1−3), thus, as expected, no gender differentiation was observed. But the synthesis of cholesterol is the key reaction in steroidogenesis, occurring in all tissues (1). After the administration of EP, serum cholesterol was increased only in females. This effect was absent in males, possibly due to the anabolic effects of androgen hormones.

These results were in agreement with those obtained following the administration of EP to young sportsmen (Table 2), where androgen hormones increased significantly. In a previous paper we showed that intensive physical effort induces an oxidative stress in young sportsmen (7). We already observed that the administration of EP significantly decreases the oxidative stress (5), improving the physical performances. Therefore, the administration of EP to young sportsmen is beneficial by providing an increased supply of androgen hormones and by its protective effect against the oxidative stress.

The most interesting results were obtained following the administration of EP to old subjects (Table 3). It is known that the genetic program of the aging process induces marked metabolic modifications (6, 8). These modifications include some pathways of steroidogenesis, such as the increase of cholesterol and cortisol and the decrease of sexual steroids. The administration of EP induces adaptive-regulatory modifications for each individual, as function of initial values. Most of the adaptive-regulatory mechanisms in the organism are triggered by the activation of hypothalamus-pituitary-adrenal (HPA) neuroendocrine axis. Indeed, our results favor an action of EP on HPA axis, directly or by intermediacy of growth factors

such as IGF-1 (Table 3). Our results support this conclusion by the regulation of 17-ketosteroids and cortisol in both sexes.

Wang and Chard showed that IGF-1 regulates the ovarian and endometrical functions in humans (9), but also steroid synthesis in adrenal gland (10–12). It is known that IGF-1 has a molecular weight of 6 kDa, very close to that of EP. IGF-1 arises from growth hormone, also secreted by the pituitary gland into the blood (3).

Since 1966, one of the authors has shown that the EP extract administered to immature bulls stimulates the sexual development (13). Our present results on humans are in agreement with the previous observations.

Steroidogenesis, regardless of the site of synthesis (adrenal or gonads), requires an energetic source provided by mitochondria (14). But mitochondria are also a powerful source for releasing oxygen reactive species (15, 16) that induce an oxidative stress, strongly associated with the aging process (6, 16). As the administration of EP significantly decreases the oxidative stress (5), a coupled effect on steroidogenesis is also possible.

Our results support an implication of neuroendocrine HPA axis in the regulation of steroidogenesis, in agreement with other data (9–12). The administration of EP might act on this mechanism, directly or by the intermediacy of growth factors such as IGF-1. A direct connection between EP and IGF is supported by the data presented in Table 3.

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