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Estrogenic Effect of Estradiol-sulfamate on the Male Rat Anterior Pituitry

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Estrogen sulfamates (ES) are used for a new treatment strategy to avoid liver–hormone and hormone–liver interactions. ES represent new synthetic steroids having an increased systemic and reduced hepatic estrogenicity when given orally [1, 2]. In the present study effects of ES and estradiol-benzoate (EB) on adenohypophyseal (AP) and serum concentrations of prolactin (PRL), luteinizing hormone (LH), and pituitary contents of cAMP and cGMP in the male rat are demonstrated. The weight gain of experimental animals treated by ES, EB or both hormones simultaneously was significantly lower compared to controls. EB but not ES significantly increased the weight of the AP. The amounts of PRL in the AP and serum were significantly increased after EB administration. ES significantly increased only AP content of PRL. EB administered simultaneously with ES exhibited an additive effect on the AP plasma concentrations of PRL. The EB or ES significantly decreased AP and serum concentrations of LH. ES given simultaneously with EB further decreased AP and serum concentrations of LH. After administration of either ES or EB, AP contents of cAMP and cGMP were significantly increased. An additive effect of these estrogens on the cGMP content was found. ES given simultaneously with EB further increased cGMP content in the AP but partially inhibited the effect of EB on the AP cAMP content. The present results demonstrate that the effects of ES on the AP content of PRL, LH, cAMP, and cGMP differ from the effects of EB. Whether this is due to lower levels of estradiol after the administration of ES secondary to its different absorption when compared to EB is unknown. Thus, our data support the concept that the ES has a lesser estrogenic effect on the AP function. © **1998 Published by Elsevier Science Ltd. All rights reserved.**

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INTRODUCTION

Estrogens are widely used, especially as oral contraceptives or hormone replacement therapy in postmenopausal women. Current available estrogens are represented by natural and synthetic types. Natural estrogens are extensively metabolized in the liver and this biotransformation decreases their therapeutic effect.

Estrogen sulfamates (ES) are new synthetic estrogens that do not undergo biotransformation in the liver and therefore may be used in much smaller doses to achieve the same therapeutic effect when compared to other currently known synthetic estrogens [1, 2, 6–9]. ES are derivates of steroid estrogens where the phenolic hydroxygroup is substituted with an aminogroup ($-O\cdot SO_2\cdot NH_2$). ES as prodrugs of their parent estrogen, do not affect any liver function during the first-pass [1] thus in this way they imitate the effects of natural estrogens given transdermally. For example, they do not affect the transcription of the estrogen-modulated angiotensin gene and high density lipoprotein levels in the liver. As described previously, estron sulfamate also inhibits sulfatase in the liver [9].

In the present study, the estrogenic effect of estradiol-benzoate (EB) and ES on the AP content of PRL, LH, and intracellular messengers cAMP and cGMP in the rats were evaluated.

MATERIAL AND METHODS

One hundred and forty male rats (Wistar, VÚFB, Konárovice), each weighting approximately 180 g were used. They were fed on a standard laboratory

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Table 1. *Changes of total body weights and anterior pituitary (AP) after chronic treatment with estradiol-benzoate (EB) and estradiol-sulfamate (ES)*

	Controls (1)	EB 0.5 mg (2)	EB 5.0 mg (3)	ES 0.45 mg (4)	ES 4.5 mg (5)	EB + ES 0.45 + 0.5 mg (6)	EB + ES 4.5 + 5 mg (7)
Animal weight (g)	252.7 ± 7.3 (2,3,4,5,6,7)	176.9 ± 12.2 (1,7)	174.1 ± 9.3 (1,7)	196.8 ± 32.8 (1,7)	169.0 ± 3.8 (1,7)	171.0 ± 8.2 (1,7)	157.4 ± 9.4 (1,2,3,4,5,6)
AP weight (mg)	8.7 ± 0.25 (2,3,5,6,7)	17.3 ± 0.7 (1,4,5)	18.3 ± 4.1 (1,4,5)	10.9 ± 1.46 (2,3,6,7)	12.1 ± 0.88 (1,2,3,6,7)	18.2 ± 2.8 (1,4,5)	16.4 ± 0.6 (1,4,5)

Mean values ±95% confidence interval. Significance of inter-group differences is given in parentheses. 20 animals in each group.

diet (Larsen diet, VELAZ), with water ad libitum. For the two experiments they were divided into several groups (ten animals per each group): controls (C, given 0.9% NaCl), estradiol-benzoate (EB, aqueous microcrystal suspension, Agofolin-Depot, Biotika, 0.5 and 5 mg/kg i.m. twice a week), estradiol-sulfamate (ES, 0.45 or 4.5 mg/kg i.m. twice a week), ES and EB given simultaneously. The animals were weighed at the beginning and at the end of the experiment. After three weeks of treatment rats were killed by decapitation. The blood was immediately collected and the AP was dissected out, weighed, and quickly frozen in liquid nitrogen. ES was prepared and purified by Dr. Pouzar (Steroid department of Academy of Sciences, Czech Republic) according to Schwarz [4, 5].

Serum concentrations and AP content of PRL and LH were determined by double antibody RIA using NIADDK kits, generously provided by Dr. Parlow. The sensitivity of RIA was 0.08 ng/ml for LH and 0.1 ng/ml for PRL. PRL, intra-assay was 9.1 ± 2.2, inter-assay was 12.3 ± 2.8, LH intra-assay was 8.6 ± 1.5 and inter-assay was 11.5 ± 2.2. cGMP and cAMP contents in the AP were measured by RIA (Immunotech, Prague). cGMP intra-assay was 7.6 ± 1.9 and inter-assay was 9.8 ± 2.4, cAMP intra-assay was 8.2 ± 2.0 and inter-assay was 12.0 ± 2.9.

The frozen AP was homogenized in a Polytron homogenizer, two times 10 s in water containing EDTA (0.5 mol/l) to prevent enzymatic breakdown of the cGMP or cAMP, followed by 3 min heating in a boiling water bath to coagulate the proteins and by 10 min centrifugation at 4°C in a Beckman type GPR centrifuge. The supernatant was collected and frozen at −20°C until analyzed. The estradiol was determined by a RIA kit (Immunotech, Prague). The detection limit was 3 pg/ml. Intra-assay and inter-assay were 6.8 ± 1.9 and 13.9 ± 2.5, respectively.

The means and the 95% confidence intervals were computed and the significance of differences between the means was calculated by an analysis of variance, using the post-hoc Student–Newman–Keuls test.

RESULTS

The total body weights of the experimental male rats treated either with EB, ES or EB + ES are summarized in Table 1. Rats treated with estrogens had significantly lower total body weight compared to controls. EB given alone significantly increased AP weight compared to controls, whereas ES given alone

Table 2. *Effect of chronic administration of estradiol-benzoate (EB), estradiol-sulfamate (ES), and EB + ES on serum and anterior pituitary contents of PRL, LH, estradiol, cAMP and cGMP*

	Controls (1)	EB 0.51 mg (2)	EB 5.0 mg (3)	ES 0.45 mg (4)	ES 4.51 mg (5)	EB + ES 0.45 + 0.5 mg (6)	EB + ES 4.5 + 5 mg (7)
PRL ng/ml serum	2.7 ± 1.4 (2,3,4,5,6,7)	21.3 ± 8.2 (1,4,7)	26.2 ± 6.5 (1,4,5,7)	7.4 ± 3.7 (2,3,5,6,7)	17.4 ± 7.8 (1,3,4,7)	22.2 ± 6.6 (1,4,7)	34.7 ± 6.5 (1,2,3,4,5,6)
PRL ng/AP	6.2 ± 4.2 (2,3,4,5,6,7)	40.5 ± 13.7 (1,4,5,6,7)	43.7 ± 19.9 (1,4,5,6,7)	20.3 ± 8.4 (1,2,3,6,7)	24.0 ± 3.9 (1,2,3,6,7)	54.9 ± 16.1 (1,2,3,4,5)	63.0 ± 20.8 (1,2,3,4,5)
LH ng/ml, serum	0.3 ± 0.03 (3,5,6,7)	0.21 ± 0.03 (1)	0.19 ± 0.02 (1,4,5)	0.23 ± 0.03 (3)	0.22 ± 0.03 (1,3)	0.2 ± 0.02 (1)	0.2 ± 0.02 (1)
LH ng/AP	1.6 ± 0.34 (2,3,4,5,6,7)	0.42 ± 0.09 (1,4,5,7)	0.45 ± 0.20 (1,4,7)	0.9 ± 0.47 (1,2,3,5,6,7)	0.33 ± 0.16 (1,2,4,7)	0.37 ± 0.15 (1,4,7)	0.20 ± 0.01 (1,2,3,4,6)
estradiol pg/ml serum	19.7 ± 6.1 (2,3,4,5,6,7)	199 ± 63 (1,3,4,5,6,7)	2527 ± 479 (1,2,4,5,6)	34.9 ± 17.5 (1,2,3,5,6,7)	97.4 ± 30.0 (1,2,3,4,6,7)	319 ± 98 (1,2,3,4,5,7)	2234 ± 316 (1,2,4,5,6)
cAMP pmol/AP	98.9 ± 31.8 (2,3,4,6,7)	416 ± 126 (1,4,5,7)	500 ± 170 (1,4,5,6,7)	157 ± 27.0 (1,2,3,6,7)	132 ± 46.5 (2,3,4,6,7)	404 ± 118 (1,4,5)	346 ± 84.6 (1,2,3,4,5)
cGMP pmol/AP	0.14 ± 0.07 (2,3,4,5,6,7)	0.67 ± 0.22 (1,4,5,7)	0.87 ± 0.42 (1,4,5,7)	0.25 ± 0.11 (1,2,3,6,7)	0.26 ± 0.06 (1,2,3,6,7)	0.97 ± 0.38 (1,4,5,7)	1.04 ± 1.13 (1,2,3,4,5,6)

Mean ± 95% confidence intervals. The number in parentheses denote groups with statistically different means (Student–Newman–Keuls Method). 20 animals in each group.

increased AP weight only slightly. When EB was given in combination with ES, total body weight was further significantly reduced compared to control rats.

Table 2 summarizes effects of EB and ES given either alone or in combination on serum concentrations or AP contents of PRL, LH, estradiol, cAMP, and cGMP. Chronic treatment with EB significantly increased serum and AP concentrations of PRL. After treatment with ES alone serum and AP concentrations of PRL decreased as well. The administration of EB + ES had an additive effect on serum and AP concentrations of PRL. Serum levels of LH were significantly decreased after EB, ES, or EB + ES treatment.

The serum levels of estradiol are summarized in Table 2. In the rats treated with EB serum estradiol increased by about 100 times, whereas in rats treated with ES serum estradiol levels increased by only about 5 times. These effects were dose-dependent.

The content of cAMP and cGMP in the AP increased after either EB or ES treatment. ES given simultaneously with EB (5 mg/kg) partially inhibited the EB-induced increase in AP cAMP. Administration of ES + EB had an additive stimulatory effect on the AP cGMP content.

DISCUSSION

The present study demonstrates that ES-induced AP growth was much smaller than after EB treatment. Furthermore, the AP function measured by adenohypophyseal tissue or serum concentrations of PRL and LH was affected differentially after ES compared to EB treatment. The AP and serum concentrations of PRL were significantly increased after EB treatment and to lesser extent after ES treatment. In doses 4.5 and 5 mg/kg, ES + EB had an additive affect on PRL concentrations in the AP. In contrast, EB more profoundly decreased AP and serum concentrations of LH compared to ES. ES + EB (4.5 and 5 mg/kg) had a marked additive effect on the AP content of LH. Thus, the present results suggest that ES affects the AP function to a lesser extent than EB. Whether this is due to different estradiol levels after either EB or ES treatment that could be result from different absorption is unknown.

After chronic treatment with EB, AP contents of cAMP and cGMP were significantly increased. ES administered simultaneously with EB inhibited the EB-induced increase in cAMP and stimulated the EB-induced increase in cGMP in the AP.

As previously reported, estrogen treatment of male rats induced an increase in anterior pituitary cGMP and cAMP content [10], and uterine [11] cGMP content. According to Galand and Rooryck [11], EGF might be an intermediate in the response of cGMP to estradiol in the uterus. Recently, we have demonstrated increased concentrations of EGF in the AP after chronic EB treatment [12]. Furthermore, it is known that cGMP is a product of NO-synthase and the NO-synthase system is present in the adenohypophysis. Recent evidence suggests that NO plays an important role in the regulation of the LH and PRL secretion [13, 14] and estrogens may modulate this effect through cGMP. To support this hypothesis, we have also recently demonstrated the regulation of the AP cGMP content after L-NAME (N-nitro-L-arginine methyl ester), an inhibitor of NO-synthase.

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