Influence of naringenin on the activity of enzymes participating in steroidogenesis in male rats

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Abstract

The aim of the experiment was to histochemically examine the activity of the following selected steroidogenic enzymes: $\Delta^5$3βHSD and 17βHSD and the housekeeping enzyme G6PDH, in experimental rats after subcutaneous injections of naringenin flavonoid, at a daily dose of 15 mg/kg of body mass. The enzyme activities were measured by the microdensitometric method. Additionally, radioimmunological assay for testosterone level was conducted in homogenates of testes. A significant decrease in the activity of 17βHSD and of G6PDH was found, while the activity of $\Delta^5$3βHSD was not significantly changed. The results permit a statement that naringenin causes minor changes in metabolic processes in the testes of rats but it does not significantly affect the synthesis of androgens.

Key words: $\Delta^5$3β-hydroxysteroid dehydrogenase, 17β-hydroxysteroid dehydrogenase, glucose-6-phosphate dehydrogenase, histochemistry, testis, rat, naringenin.

Introduction

Naringenin is a biphenolic compound, belonging to the group of flavonoids and to the class of flavanones. This flavonoid occurs in greatest quantities in citrus fruits, as well as in hop [1]. Because of its affinity to the estrogen receptor α and the ability to stimulate proliferation of cells in female reproductive tracts, this flavonoid is placed among the phytoestrogens, i.e., non-steroid compounds of plant origin, showing a structural similarity with 17β-estradiol [2, 3]. Like other phytoestrogens, naringenin inhibits the activities of 17βHSD and aromatase *in vitro* [4]. The objective of this study was to examine the *in vivo* effects of naringenin on the activity of selected steroidogenic enzymes: $\Delta^5$3βHSD and 17βHSD. as well as G6PDH, a housekeeping enzyme, providing the NADPH necessary for the synthesis of steroids. The studies were combined with an analysis of testosterone level.

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Material and Methods

The experiments involved 20 sexually mature male rats of the inbred Wistar strain. The animals were kept in standard conditions: temperature 22°C, air humidity 50-60%, and the 12/12 hours L/D light regime. The animals were fed with standard fodder and had tap water available *ad libitum*. The rats were divided into two groups of 10 animals in each: control and experimental. The experimental rats received naringenin subcutaneously at a dose of 15 mg/kg b.m. in 0.2 ml dimethylsulphoxide (DMSO) each day for 14 consecutive days. The control rats were given subcutaneous injections of DMSO. After 14 days, the rats were decapitated and their testes removed and frozen in liquid nitrogen. The testes were then cut into 8 μm sections in a cryostat and subjected to histochemical reactions to detect $\Delta^5$3βHSD (EC 1.1.1.145) [5], 17βHSD (EC 1.1.1.51) [6,7], and G6PDH (EC 1.1.1.49) [8]. The enzyme activity in Leydig cells was assessed by the microdensytometric method. A computer-assisted image analyser, with the Multi Scan 6.08 software and an 8-bit grey scale, was used for the assessment. The intensity of the histoenzymatic reaction was estimated by measuring the integrated optical density (IOD) in the marked area. The intensity of histochemical reactions, shown on microphotographs, was assessed as either weak, moderate, or strong.

A portion of the material was placed aside to carry out a radioimmunological assay for testosterone level. This determi-

nation was completed with the use of a standard kit (from DSL) [9]. The significant differences between the groups were statistically tested, using ANOVA procedure.

Results

The greatest intensity of enzymatic reactions occurred in Leydig cells. The activity in the seminiferous epithelium was either weak or absent, depending on the examined enzyme.

The activity of $\Delta^5$3βHSD in Leydig cells of the control rats was strong (Fig. 1a), whereas in the rats, treated with naringenin, it was either high or moderate. The activity of 17βHSD in Leydig cells of the control rats was moderate, while the intensity of that reaction in the experimental rats was weak (Fig. 1 b,c). The activity of G6PDH in Leydig cells of the control rats was either moderate or high, whereas histochemical reaction in the experimental animals was moderate (Fig. 1d,e). Microdensitometric measurements showed a statistically significant decrease in 17βHSD and G6PDH activities in the experimental rats, compared with the respective values in the control animals (Fig. 2). The difference between the control and the experimental group in the $\Delta^5$3βHSD was not significant. In the homogenates of testes of the experimental rats, a statistically insignificant increase in testosterone content (120 ± 45 ng/g tissue) was found, compared with that in the control group (82 ± 26 ng/g tissue).

Discussion

This paper presents preliminary results of a study on the effects of naringenin *in vivo*, on the activity of selected enzymes, involved in the biosynthetic function of rat testes.

The observed drop of 17βHSD activity in the rats, receiving naringenin, may indicate a hormone-like effect of this flavonoid. It is known from literature that the administration of 17β-estradiol to male rats also leads to a drop in steroidogenic enzymes [10]. The obtained results match those of Le Bail et al. [4], who demonstrated that naringenin inhibited the activity of 17βHSD and aromatase in the microsomes, isolated from the human placenta. The application of naringenin to the rats did not result in any changes in the activity of 3βHSD, compared to respective values in the control. The results again agree with those, reported by Le Bail et al. [11], who demonstrated that naringenin in vitro had not affected the activity of 3βHSD, an enzyme, involved in steroidogenesis. Despite the drop in 17βHSD activity, in the rats receiving naringenin, a slight increase in testosterone level occurred in the testes of those individuals. One may thus presume that, in rats, naringenin inhibits the activity of the enzymes, participating in the metabolism of testosterone, such as aromatase and 5α-reductase. It should be added that the effect, exerted by phytoestrogens on the synthesis and release of androgens, has not yet been determined and the results of studies often contradict one another [12, 13]. In the rats, receiving naringenin, a decrease in the level of anabolic processes was noted, as indicated by a drop in the activity of G6PDH dehydrogenase. The decrease in

Figure 1. (a) Histochemical reaction for $\Delta^5$3βHSD (200x). Strong reaction in Leydig cells (arrow) of a control rat. (b,c) Histochemical reaction for 17βHSD (320x). The arrows show Leydig cells. (b) strong reaction in Leydig cells of a control rat. (c) Moderate and weak reaction in Leydig cells of an experimental rat. (d,e) Histochemical reaction for G6PDH (200x). (d) Moderate reaction in Leydig cells (arrow) of a control rat. (e) Weak reaction in Leydig cells (arrow) of an experimental rat.

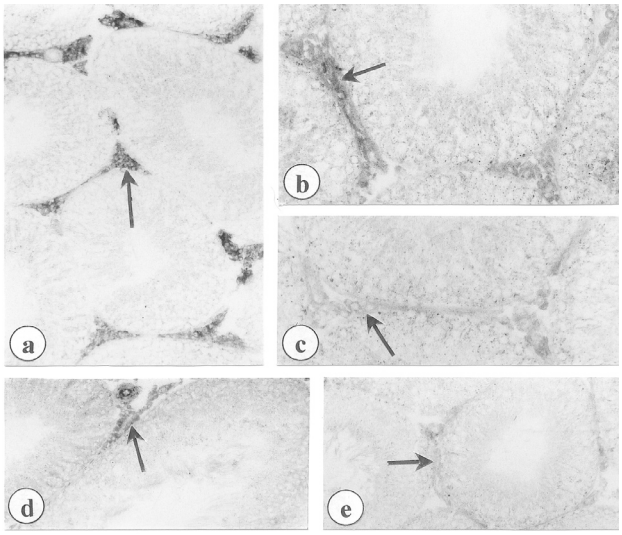
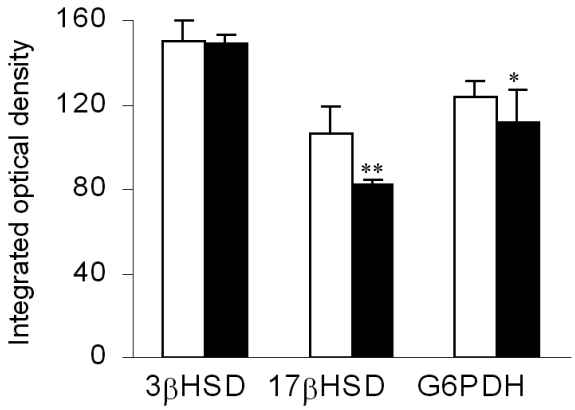


Figure 2. The activity of dehydrogenases in Leydig cells of the control and naringenin treated rats. Microdensytometrical analysis. Values are the means ± SD. Statistically significant differences as compared to the controls. *p<0.05, **p<0.01.



G6PDH was also observed after the administration of steroidogenesis-inhibiting chemicals [14]. The results of the present study allow for a statement that naringenin is an inhibitor of 17βHSD and G6PDH in the testes of rats and does not significantly affect the activity of $\Delta^5$3βHSD or the synthesis of testosterone.

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