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The Effect of Administration of Estradiol and Testosterone on Body Growth of Young Male Rats

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Summary

The influence of estradiol and testosterone on body growth of young male Wistar rats was investigated. In the first experiment, estradiol was given to intact *ad libitum* fed male rats at 32, 37 and 42 days of age. Moreover, two untreated groups of animals were used: one was fed restrictedly according to the food intake of animals receiving estradiol and another was fed *ad libitum*. The animals were sacrificed at 47 days of age. Both untreated groups of animals achieved significantly higher body weight and length of tibia than estradiol treated animals. Also the growth of the tail of untreated animals was more intensive than that of estradiol treated animals. In the second experiment, estradiol was given to intact *ad libitum* fed male rats at 30, 35 and 45 days of age. Moreover, testosterone was given to a half of these animals at 45, 50 and 55 days of age. The animals were sacrificed at 60 days of age. Administration of testosterone significantly increased the growth of the tail and tibia in comparison to the animals which did not receive testosterone after estradiol administration. The results of the present study show that the inhibitory effect of estradiol on body growth of young male rats is not only the result of decreased food intake and that testosterone can improve the skeletal growth of male rats altered by previously given estradiol.

Key words

Body growth - Estrogens - Androgens

Introduction

The sexual dimorphism in body growth of males and females in many mammalian species, including rats, is conditioned mainly by the presence of different gonadal steroids – androgens and estrogens. The significance of androgens and estrogens in growth regulation is confirmed by the consequences of the removal of main endogenous sources of these hormones. Gonadectomy of male rats decreases body growth and food intake (Kakolewski *et al.* 1968, Gentry and Wade 1976, Jansson *et al.* 1984) and replacement treatment with androgens, in adequate doses, increases both (Gray *et al.* 1979, Rowland *et al.* 1980, Jansson *et al.* 1983). Gonadectomy of female rats increases body growth and food intake (Kakolewski *et al.* 1968, Clark and Tarttelin 1982, McElroy and Wade 1987) and replacement treatment with estrogens decreases both (Wade 1975, Jansson *et al.* 1983, Gavin *et al.* 1984). Administration of androgens to intact male or female rats can stimulate body growth (Rubinstein and Solomon 1941, Kochakian *et al.* 1950, Tarttelin *et al.* 1975, Kuchár *et al.* 1982). On the other hand, administration of a high dose of testosterone reduces the growth in both intact and gonadectomized rats (Kochakian and Endahl 1959, Joss *et al.* 1963). This effect is assumed to be caused by the aromatization of testosterone to an estrogen (Gentry and Wade 1976, Gray *et al.* 1979). Administration of estrogens to intact male or female rats decreases body growth and food intake (Dubuc 1976, Kuchár *et al.* 1982, Finkelstein 1986). However, the administration of a low single dose of estradiol (as well as the administration of testosterone) in early postnatal life stimulates body weight of female rats at later periods of life (Bell and Zucker 1971, Donohoe and Stevens 1983). On the other hand, neonatal administration of a high single dose of estradiol retards the growth of female as well as male rats later in life (Ošťádalová and Pařízek 1968, Ošťádalová *et al.* 1969, Ošťádalová 1976).

The aim of the present study was to investigate whether the decrease of the body growth of young male rats after estradiol administration is only the result of decreased food intake and whether the administration of testosterone can improve the growth of male rats altered by previously given estradiol.

Materials and Methods

Animals

Postweaning male Wistar rats were used in experiments. The animals were housed in plastic cages (5 in each) in a temperature controlled environment of 22 °C with 12:12 hs light–dark regime. The animals had free access to standard laboratory diet DOS 2b (except those fed restrictedly) and to tap water.

First experiment

Thirty postweaning male rats were divided into 3 groups. At 32, 37 and 42 days of age the long-acting estradiol preparation Agofolin Depot (oestradiolum benzoicum in susp. aq., Biotika, Czechoslovakia; 0.25 mg per animal) was subcutaneously given to the group designated E. 150 mM NaCl, in the same volume as estradiol, was given to the groups designated C (control) and RF (restrictedly fed) on the above mentioned days. The body weight of each individual animal and the collective food intake of each group (food intake per cage) was measured daily. The food intake of each group was determined for each 24 hour's period of the experiment in grams per kg of initial body weight (i.e. the body weight at the beginning of an appropriate 24 hours' period). The restrictedly fed animals received the same amount of food (in g/kg) as the estradiol treated animals one day earlier (the restrictedly fed animals were one day younger than the estradiol treated animals). The efficiency of food utilization (EFU) of each group of animals was determined for each 24 hours' period of the experiment: the body weight gain for 24 hours was divided by food intake in the same period. The tail length (from the anus to the tip of the tail; under ether anaesthesia) was measured at 32 and 47 days of age. The animals were sacrificed at 47 days of age and the tibia was dissected out for the determination of its length.

Second experiment

Thirty postweaning male rats were divided into 3 groups. At 30, 35 and 40 days of age the long-acting estradiol preparation Agofolin Depot (oestradiolum benzoicum in susp. aq., Biotika, Czechoslovakia; 0.25 mg per animal) was subcutaneously administered to the groups designated E and ET. Moreover, the long-acting testosterone preparation Agovirin Depot (testosteronum isobutyricum in susp aq., Biotika, Czechoslovakia; 1.25 mg per animal) was given to the group ET at 45, 50 and 55 days of age. 150 mM NaCl, in the same volume as the hormones, was given to the control group (designated C) on the above mentioned days. At 30, 45 and 60 days of age the body weight and the tail length (from the anus to the tip of the tail; under ether anaesthesia) were measured. 72 and 24 hours before killing oxytetracycline (Oxymykoin, SPOFA, Prague; 10 mg/kg of body weight, intraperitoneally) was given for determination of the rate of longitudinal growth of the tibia by the tetracycline method (Hansson 1967). At 60 days of age the animals were sacrificed and the tibia was dissected out for determination of its length, thickness of proximal growth plate and rate of longitudinal growth.

Determination of the rate of longitudinal growth of tibia from the proximal growth plate

The proximal end of the tibia was dissected free and cut with a blade between condylus medialis and condylus lateralis and then fixed in formalin for 24 hours. 80 lm thick longitudinal sections were made on a cryostat. The sections were dehydrated in ethanol and cleared in xylene. Finally, the sections were mounted in BVX and photographed under a fluorescencent microscope (Jenalumar contrast, Carl Zeiss Jena) together with a slide equipped with a micrometer 1 mm long and graduated into 100 parts. The distance between two fluorescent oxytetracycline labels was measured on the photographs. The thickness of the proximal growth plate was measured in the same sections under a light microscope with an ocular equipped with a micrometer.

The results were statistically evaluated by ANOVA and Duncan's test.

Results

First experiment

The mean daily food intake (g/kg of body weight) between the 32nd and 47th day of age was 19 % lower in E and RF animals than in control animals (Tab. 1). The mean daily efficiency of food utilization (mg of weight gain/g of received food) between the 32nd and 47th day of age was 29.9 % and 10.8 % lower in E and RF animals than in the controls, respectively (Tab. 1).

The body weight did not differ significantly between individual groups of animals at 32 days of age. The body weight of control animals was significantly higher than that of E and RF animals from the 36th day of age ($p<0.01$), moreover, it was significantly ($p<0.01$) higher in RF animals than in E animals at 46 and 47 days of age (Fig. 1, Tab. 2). The total body weight gain of the groups between the 32nd and 47th day of age is shown in Table 1.

The tail length did not differ significantly between individual groups of animals at 32 days of age. At the end of the experiment (the 47th day of age) it was significantly higher in controls than in E and RF animals (Tab. 2). The tail length of E and RF animals did not differ significantly at the end of the experiment

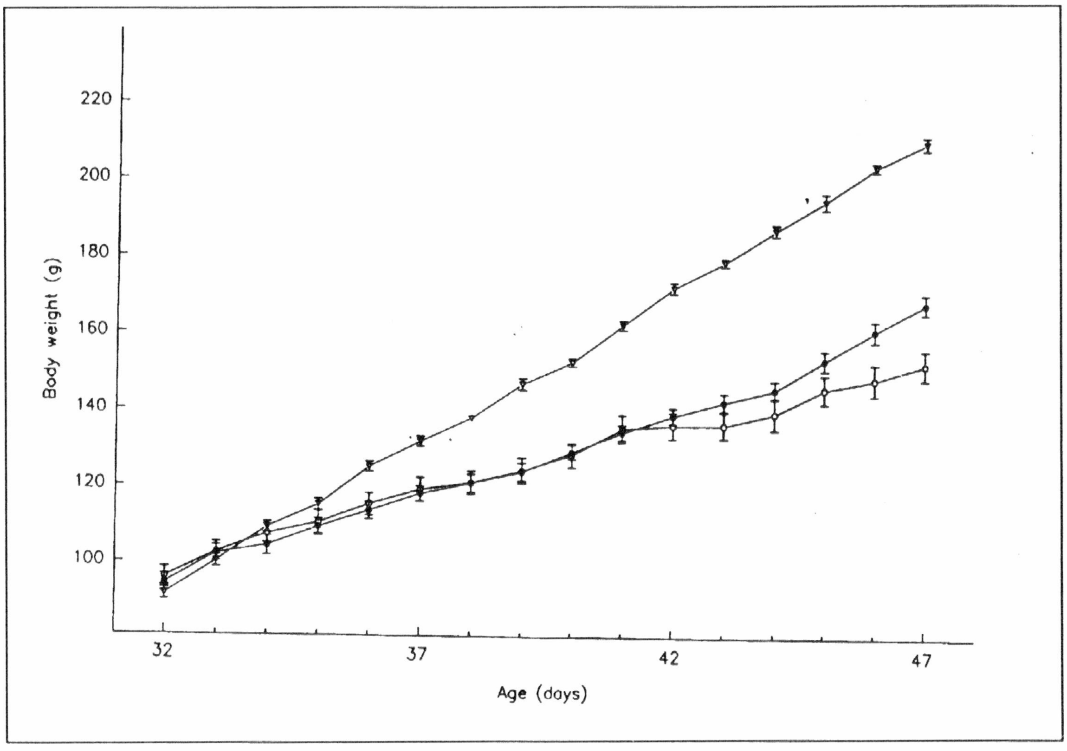

Fig. 1
Body weight of estradiol treated (open circles), restrictedly fed (full circles) and control (triangles) male rats. Values are means ± S.E.M.

Table 1
Mean daily food intake and efficiency of food utilization (EFU), total weight and tail length gain between the 32nd and 47th day of age in control (C), estradiol treated (E) and restrictedly fed (RF) animals

	C	E	RF
Food intake (g/kg)	132.0	107.0	107.0
EFU (mg/g)	411.0	288.2	366.6
Total weight gain (g)	119.2 ± 0.75^a	56.60 ± 1.47^b	74.2 ± 0.83^c
Total tail length gain (mm)	53.1 ± 0.74^a	33.70 ± 0.49^b	37.8 ± 0.80^c

Values are given as mean daily food intakes and EFUs in each experimental group or as means of total body weight and tail length gains of individual animals ± S.E.M. Values with different superscripts in one line are significantly different from each other ($p<0.01$)

(Tab. 2), however, the total tail length gain between the 32nd and 47th day of age was significantly higher in RF animals than in E animals (Tab. 1).

There were also significant differences in tibia length between separate groups of animals at the end of the experiment (Tab. 2).

Second experiment

Body weight and tail length did not differ significantly between individual groups of animals at the beginning of the experiment (the 30th day of age). At the end of the experiment (the 60th day of age), the E and ET animals had a significantly lower body weight, tail length and tibia length than the control animals (Tab. 3). Body weight and tail length did not differ significantly between E and ET animals at the end of the experiment, but there was a significant difference in the length of the tibia between these two groups of animals (Tab. 3).

The body weight gain between the 30th and 45th day of age was significantly ($p<0.01$) lower in both

Table 2
Body weight, tail length and tibia length on the 47th day of age in control (C), castradiol treated (E) and resctrictedly fed (RF) animals

	C	E	RF
Body weight (g)	213.6 ± 3.28^a	152.7 ± 3.83^b	168.6 ± 2.60^c
Tail length (mm)	169.4 ± 2.70^a	151.0 ± 1.56^b	153.6 ± 1.26^b
Tibia length (mm)	33.8 ± 0.25^a	31.4 ± 0.31^b	32.6 ± 0.04^c

Values are means ± S.E.M. Values with different superscripts in one line are significantly different from each other ($p<0.01$)

groups of animals receiving estradiol (E and ET) than in control animals, but no significant differences were found between these three groups of animals in the following period of the experiment (45–60th day of age). The tail length gain was significantly ($p<0.01$) higher in control animals than in the other two groups in both estimated periods of the experiment (i.e. 30–45th and 45–60th day of age). The tail length gain of E and ET animals did not differ significantly in the first period of the experiment but in the second period (i.e. after testosterone administration) it was significantly ($p<0.01$) greater in ET animals than in E animals.

The thickness of the proximal growth plate of the tibia at the end of the experiment and the rate of longitudinal growth of tibia from the proximal growth plate between the 57th and 59th day of age were the greatest in the control animals, smaller in the ET animals and the least in the E animals, but these differences were not significant (Tab. 3).

Table 3
Body weight, tail length, tibia length and thickness of proximal growth plate of the tibia (TGP) on the 60th day of age and the rate of longitudinal growth of tibia (RGT) between the 57th and 59th day of age in control (C), estradiol treated (E) and estradiol+testosterone treated (ET) animals

	C	E	ET
Body weight (g)	309.2 ± 15.90^a	256.6 ± 9.22^b	266.2 ± 3.54^b
Tail length (mm)	204.3 ± 2.28^a	189.3 ± 0.50^b	193.2 ± 1.65^b
Tibia length (mm)	38.3 ± 0.48^a	35.5 ± 0.20^b	36.6 ± 0.13^c
TGP (lm)	346.8 ± 10.02^a	316.5 ± 8.55^a	333.6 ± 11.90^a
RGT (lm/48 h)	392.5 ± 20.16^a	369.5 ± 15.90^a	387.3 ± 11.70^a

Values are means ± S.E.M. Values with different superscripts in one line are significantly different from each other ($p<0.01$)

Discussion

The results clearly show an inhibitory effect of estradiol on body weight and food intake as well as on skeletal growth of young male rats in agreement with the data of other authors (Dubuc 1976, Kuchár *et al.* 1982). The body weight gain of estradiol treated animals was lower by 52.5 % than that of untreated controls in the present study. These results are comparable with other data (Kuchár *et al.* 1982) demonstrating that the body weight gain of estradiol treated male rats of similar age as in the present study decreased approximately by 50 %. In older animals, a loss of body weight was found after estradiol administration (Kuchár *et al.* 1982). The untreated animals which received the same amount of food as estradiol treated animals (i.e. the restrictedly fed animals) achieved significantly a higher body weight gain than estradiol treated animals in the present study. However Sullivan and Smith (1957) obtained parallel growth curves (body weight) in estradiol treated and pair-fed young male rats (weighing less than 80 g at the start of the experiment). On the other hand, Glasser (1954) demonstrated almost 2 times greater loss of body weight in adult male rats receiving the diethylstilbestrol in comparison with pair fed animals. Moreover, Josimovich *et al.* (1967) showed that the inhibitory effect of estradiol on the growth hormone induced widening of the tibial growth plate in hypophysectomized female rats occurred independently

of the estradiol effects on food intake. This is in agreement with the results of the present study showing better skeletal growth of restrictedly fed animals in comparison to estradiol treated animals. Thus, our results support the opinion that the anorexic effects of estradiol cannot completely explain the reduction of body weight (Roy and Wade 1977, Simpkins *et al.* 1988).

The fact that the efficiency of food utilization in estradiol treated animals is impaired may partly explain the differences in body growth between these animals and untreated ones. Estrogen treatment of male rats results in a pattern of growth hormone secretion similar to that found in intact female rats (Mode *et al.* 1982). These results indicate that estradiol administered to male rats can depress the body growth not only by decreasing food intake but probably also by influencing the growth hormone secretory pattern.

Our results show that the administration of testosterone improved the skeletal growth of male rats altered by previously given estradiol. There was only a nonsignificant difference between estradiol treated and control animals in the rate of longitudinal growth of the tibia between the 57th and 59th day of age. This result indicates that estradiol already did not have a strong influence on tibia growth in this period (the last dose of estradiol was given 17 days previously). The comparison of the rate of longitudinal growth of the tibia between estradiol + testosterone treated and estradiol treated animals showed that testosterone no longer had a strong influence on tibia growth between the 57th and 59th day of age. This result indicates that testosterone was effective mainly at the beginning of the treatment period (from the 45th day). At a later period of life the level of endogenously produced testosterone in male rats rapidly rises (Knorr *et al.* 1970, Brown-Grant *et al.* 1975) and it can probably eliminate the effects of injected testosterone.

The presence of testosterone is necessary for the induction of a normal male growth hormone secretory pattern (Jansson *et al.* 1987). Jansson *et al.* (1983) showed that the administration of testosterone to gonadectomized male rats increased the growth of long bones but this treatment was without effect in gonadohypophysectomized animals. The administration of testosterone to gonadally intact hypophysectomized male rats was also without effect on bone growth (Simpson *et al.* 1944). It indicates that testosterone exerts its stimulatory effect on body growth mainly by altering the secretory pattern of the growth hormone (Jansson *et al.* 1983). We assume that the influence of administered testosterone on secretion of the growth hormone could contribute to the improved skeletal growth of estradiol treated male rats in the present study; this influence could be due to faster restoration of a normal male growth hormone secretory pattern (which could be changed by estradiol), in comparison to animals which did not receive testosterone after estradiol administration.

The results of the present study show that the decreased food intake is not the only cause of decreased body growth after estradiol administration and that testosterone can improve the skeletal growth of male rats altered by previously given estradiol.

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