Physiol. Res. 43: 141 – 143, 1994

SHORT COMMUNICATION

The Longitudinal Growth of Tibia in Oestradiol-Treated and Restrictedly Fed Immature Male Rats

Š. ČÍKOŠ, S. KUCHÁR, J. KOPPEL

Institute of Animal Physiology, Slovak Academy of Sciences, Košice, Slovak Republic

Received September 6, 1993
Accepted December 12, 1993

Summary

The effect of oestradiol administration and restricted feeding on longitudinal tibia growth was investigated in immature male rats. The restrictedly fed animals had a significantly longer tibia, greater thickness of the growth plate, faster rate of longitudinal tibial growth as well as the greater rate of [methyl-^{3}H]thymidine incorporation into the growth plate of the tibia compared with oestradiol-treated animals. The results indicate that, in immature male rats, exogenous oestradiol can decrease the longitudinal growth of the tibia (at least partly due to inhibition of cell proliferation in the growth plate) independently of its anorexic effect.

Key words

Tibia growth – Oestradiol – Food intake – Rat

Oestrogens exert inhibitory effects on body growth and food intake in rats (Wade 1975, Jansson *et al.* 1983, Dubuc 1976). Some findings indicate that the inhibitory effect of oestrogens on body weight of adult male or female rats can not be explained by their anorexic effect only (Roy and Wade 1977, Simpkins *et al.* 1988). On the other hand, the results of Sullivan and Smith (1957) indicate that the anorexic effect of oestradiol may be the main reason for decreased body weight in immature male rats after the administration of this hormone. Nevertheless, immature male rats, which were restrictedly fed according to oestradiol-treated animals, achieved significantly higher body weight and length of the tibia than the oestradiol-treated animals in our previous work (Číkoš *et al.* 1992). To obtain more information about skeletal growth we extended our previous experiment and investigated the longitudinal growth of the tibia in oestradiol-treated and restrictedly fed male rats.

Thirty male Wistar rats divided into 3 groups (10 animals in each group housed in one cage) were used in our experiment. The animals, except of restrictedly fed ones, had free access to a standard laboratory diet (Velaz/Altromin 1520, Velaz, Prague, Czech Republic) and to tap water.

The long-acting oestradiol preparation Agofolin Depot (oestradiolum benzoicum Biotika, Prague, 0.25 mg per animal, s.c.) was administered to the first group at the age of 32, 37 and 42 days. The other two groups of animals (those fed *ad libitum* or restrictedly fed animals) received an injection of an identical volume of 150 mM NaCl at the same time as the oestradiol-treated animals. All animals were killed at 47 days of age. The body weight and tail length of each individual animal were measured at 32 and 47 days of age. The body weight and food intake of whole groups (i.e. the body weight and food intake per cage) were measured daily from the 32nd to the 47th day of life. The food intake of each group was determined for each 24-hour period of the experiment in grams per kg of initial body weight (i.e. the body weight at the beginning of the appropriate 24-hour period). The restrictedly fed animals received the same amount of food (in g/kg) as the oestradiol-treated animals on the preceding day (the restrictedly fed animals were one day younger than the oestradiol-treated animals). Sixty-nine and twenty four hours before killing oxytetracycline (Oxymykoin, SPOFA, Prague) (10 mg/kg of body weight, i.p.) was given for determination of the rate of longitudinal growth of the tibia from the proximal growth plate by the tetracycline

method (Hansson 1967). One hour before killing [methyl-^{3}H]thymidine (1 μCi/g body weight, i.p., ÚVVR, Prague) was administered for determining the rate of DNA synthesis (proliferative activity) in the proximal growth plate of the tibia. Scintillation counting of the growth plate homogenate was performed on a Beckman LS 3801 liquid scintilator and DNA was determined by a modified fluorometric method (Koppel *et al.* 1981). The radioactivity was expressed as desintegrations per minute (DPM) per microgram of DNA.

The results are given as means ± S.E.M. Statistical significance of the differences between the means was determined by ANOVA and Duncan's test.

Table 1
Length of the tibia (LT), thickness of its proximal growth plate (TGP), rate of incorporation of [methyl-^{3}H]thymidine into the proximal growth plate of the tibia (RI) on the 47th day of age and rate of longitudinal growth of the tibia from the proximal growth plate (RGT) between 69 and 24 hours before killing in oestradiol-treated animals (E), restrictedly fed animals (RF) and *ad libitum* fed controls (C).

	E	RF	C
LT (mm)	32.9 ± 0.18^a	33.9 ± 0.22^b	34.8 ± 0.19^c
TGP (μm)	238 ± 12.2^a	430 ± 23.8^b	487 ± 20.1^b
RI (DPM/μg DNA)	979 ± 31.7^a	1538 ± 50.4^b	1230 ± 99.4^c
RGT (μm/45 h)	342 ± 19.4^a	478 ± 4.37^b	524 ± 14.7^b

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

The body weight and tail length did not differ significantly between separate groups of animals at the beginning of the experiment (the 32nd day of age). Similarly as in our previous experiments (Číkoš *et al.* 1992), they were greatest in *ad libitum* fed controls, lower in restrictedly fed animals and lowest in oestradiol-treated animals at the end of the experiment (the 47th day of age), the differences being significant ($p<0.01$). The length of the tibia (LT), thickness of its proximal growth plate (TGP), rate of incorporation of [methyl-^{3}H]thymidine into the proximal growth plate of the tibia (RI) on the 47th day of age as well as the rate of longitudinal growth of tibia (RGT) between 69 and 24 hours (i.e. during 45 hours) before killing were significantly higher in untreated (*ad libitum* as well as restrictedly fed) animals than in oestradiol-treated animals (Table 1). The length of the tibia in *ad libitum* fed controls was significantly greater than restrictedly fed animals, but the TGP and RGT did not differ significantly between these two groups; the RI was even significantly higher in restrictedly fed animals than in *ad libitum* fed controls (Table 1). The mean daily food intake of oestradiol-treated (and restrictedly fed) animals was lower by 24 % than in *ad libitum* fed controls. However, in the last three 24-hour intervals of the experiment, the food intake of oestradiol-treated (and the supply of food of restrictedly fed) animals rose to the level of *ad libitum* fed controls which was probably caused by a decline in the efficacy of oestradiol to inhibit food intake at the end of the experiment.

The results of the present study support the assumption that, in immature male rats, the decreased food intake is not the only cause of decreased body growth after oestradiol administration. The difference between our results and those of Sullivan and Smith (1957), who obtained parallel growth curves in immature male rats receiving oestradiol and in pair-fed controls, can be caused by differences in body weight of animals used in the experiments. The animals used by Sullivan and Smith were about 50 g smaller than animals in our experiments. This suggests that, at lower body weight (or in younger animals), decreased food intake after oestradiol treatment may be the main reason for decreased body weight of male rats, whereas in animals with higher body weight (or in older animals) other effects of oestradiol can also be important. Furthermore, this assumption is supported by the results of our previous work (Číkoš *et al.* 1992), where the body weight (measured daily) was approximately the same in oestradiol-treated immature male rats as in restrictedly fed animals until the animals achieved body weight of about 130 g (9 days after the first hormone administration); later it became significantly higher in restrictedly fed animals than in oestradiol-treated animals.

Restricted feeding decreased the body weight, tail length and tibia length in the present study. Dearden and Mosier (1974) showed that fasting impairs cell division in the growth plate of the rat tibia. But the proliferative activity of cells in the growth plate of tibia (measured at the end of the experiment) was significantly higher in restrictedly fed animals than in *ad libitum* fed controls. This was probably caused by increasing the food supply in restrictedly fed animals to the level of *ad libitum* fed controls at the end of the experiment (according to the food intake of oestradiol-treated animals) which led to catch-up growth of the tibia. The insignificant differences in the thickness of tibial growth plate and the rate of longitudinal growth of the tibia at the end of the experiment between restrictedly fed and *ad libitum* fed controls also suggest the presence of catch-up growth in restrictedly fed animals. This catch-up growth was not found in oestradiol-treated animals; the proliferative activity in the growth plate, its thickness and the rate of longitudinal growth of the tibia at the end of the experiment were significantly lower than in *ad libitum* fed (as well as restrictedly fed) controls. Thus, the

increase of food intake in oestradiol-treated animals to the level of *ad libitum* fed controls (at the end of the experiment) did not increase tibial growth to the level of the *ad libitum* fed controls.

To conclude, the results of the present study indicate that, in immature male rats, exogenous oestradiol can retard the longitudinal growth of the tibia (at least partly by inhibiting of cell proliferation in the growth plate) independently of its anorexic effect.

Acknowledgement
This work was supported by grant 61/91 from the Slovak Academy of Sciences.

References

ČÍKOŠ Š., KUCHÁR S., KOPPEL J.: The effect of administration of estradiol and testosterone on body growth of young male rats. *Physiol. Res.* **41**: 387–392, 1992.

DEARDEN L. C., MOSIER H. D. Jr.: Growth retardation and subsequent recovery of the rat tibia, a histochemical, light, and electron microscopic study. II. After fasting. *Growth* **38**: 277–264, 1974.

DUBUC P. U.: Prepubertal estrogen treatment and somatic growth in rats. *Endocrinology* **98**: 623–629, 1976.

HANSSON L. L.: Daily growth in length of diaphysis measured by oxytetracycline in rabbit normally and after medullary plugging. *Acta Orthoped. Scand.* Suppl. **101**, 1967.

JANSSON J. O., EDÉN S., ISAKSSON O.: Sites of action of testosterone and oestradiol on longitudinal bone growth. *Am. J. Physiol.* **244**: E135–E140, 1983.

KOPPEL J., KUCHÁR S., MÓZEŠ Š., BOĎA K.: Nucleic acids and protein content of some fetal and neonatal rat organs after insulin treatment. *Endokrinologie* **80**: 294–298, 1981.

ROY E. J., WADE G. N.: Role of food intake in oestradiol-induced body weight changes in female rats. *Horm. Behav.* **8**: 265–274, 1977.

SIMPKINS J. W., ANDERSON W. R., DAWSON R., SETH A., BREWSTER M., ESTES K. S., BODOR N.: Chronic weight loss in lean and obese rats with a brain-enhanced chemical delivery system for estradiol. *Physiol. Behav.* **44**: 573–580, 1988.

SULLIVAN L. W., SMITH T. C.: Influence of estrogens on body growth and food intake. *Proc. Soc. Exp. Biol. Med.* **96**: 60–64, 1957.

WADE G. N.: Some effects of ovarian hormones on food intake and body weight in female rats. *J. Comp. Physiol. Psychol.* **88**: 183–193, 1975.

Reprint Requests
Dr. Š. Číkoš, Institute of Animal Physiology, Palackého 12, 040 01 Košice, Slovak Republic.