Physiol. Res. 41: 393 - 398, 1992

Different Effects of Oestradiol Benzoate and Norethisterone on the Blood Flow and Mineral Content in Rat Bones

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Received May 20, 1992
Accepted August 17, 1992

Summary

In three experiments (2 on famales,1 on males),we determined the blood flow in the tibia and the distal part of the femur, together with cardiac output (by means of ^{85}Sr-microspheres),tibial bone density and tibial ash weight related to bone volume. We found that 1) the bone blood flow always fell significantly after oestradiol benzoate, 2) no change occurred after norethisterone in doses corresponding to those of oestradiol benzoate, but the blood flow showed a tendency to fall after doses one order higher (it decreased significantly in one case only), 3) the density of the tibia and tibial ash weight related to bone volume rose nonsignificantly after oestradiol benzoate, but fell (mostly statistically significantly) after norethisterone. The lowering of the bone mineral indexes in rat bones after norethisterone is a surprising and potentially significant finding requiring further verification.

Key words
Estradiol – Norethisterone – Bone blood flow – Rat

Introduction

The blood flow in rat bones is significantly dependent on the sex hormones, it increases after oophorectomy and orchidectomy (Schoutens *et al.* 1984, Verhas *et al.* 1986, Kapitola *et al.* 1991) and diminishes after oestrogens, in both males and females (Kapitola and Kubíťková 1990, Kapitola *et al.* in press). The mechanisms of these local circulatory reactions are still unknown.

Some observations hint at certain possibilities in this respect. Associations have been demonstrated between the bone blood circulation and the number and the activity of the osteoblasts: correlation of the blood flow with the work of the osteoblasts (Reeve *et al.* 1988), a greater blood flow at sites with a high osteoblast concentration (Whiteside *et al.* 1977) and a raised skeletal blood flow in osteomalacia with high osteoblastic activity borne out by an increase in alkaline phosphatase activity (Tellez *et al.* 1983). We attempted to verify the possible association between osteoblast function and the bone blood flow indirectly, on the basis of clinical observations of the different effects of oestradiol and norethisterone on the osteoblasts. Norethisterone is a synthetic gestagen with partial oestrogenic and androgenic activity (Štěpán *et al.* 1989), used also in the prevention and treatment of postmenopausal bone loss (Nordin 1979, Lindsay *et al.* 1978, Abdalla *et al.* 1985). In women who had undergone oophorectomy, Štěpán *et al.* (1989) found a drop in the osteocalcin and bone alkaline phosphatase isoenzyme level after oestradiol, but not after norethisterone. Similarly, Johansen *et al.* (1990), in postmenopausal patiens, recorded a decrease in alkaline phosphatase activity during isolated oestrogen treatment, but an increase if norethisterone was administered simultaneously, a similar effect of progestagen treatment was also described by Christiansen *et al.* (1985).

The above findings gave rise to the idea that the similarly different action of oestradiol and norethisterone on the bone blood circulation might be indirect evidence of the possible participation of osteoblasts in the regulation of bone blood flow. The aim of this study was to compare the effects of oestradiol benzoate and norethisterone and thereby contribute to the unresolved question of factors regulating the bone blood flow in rats.

Material and Methods

The experimental animals were 91 rats (65 females, 26 males) (Velaz, Czechoslovakia), housed in a room maintained at 23 °C on 12-h light/dark cycles,

Table 1
Blood flow in the tibia and distal femur of female rats after the administration of long-acting oestradiol benzoate (1 mg s. c. once every 5 days) and norethisterone in a roughly corresponding dose (0.002 % in the food).

Experiment A.

Group	Controls	EB	NE
Number of rats	9	8	10
Weight of rats (in g)	276 ± 7	232 ± 5*	256 ± 5*
Blood flow in $(ml.min^{-1}).g^{-1}$:			
tibia	0.225 ± 0.023	0.119 ± 0.015*	0.198 ± 0.024
distal femur	0.322 ± 0.031	0.236 ± 0.032*	0.304 ± 0.032
Cardiac output (in $ml.min^{-1}$)	79.2 ± 5.1	64.7 ± 6.1*	64.9 ± 5.6*
Cardiac output in $(ml.min^{-1}).100g^{-1}$	28.7 ± 1.7	28.0 ± 2.6	25.2 ± 1.9

Means ± standard errors of the means.
Statistically significant differences ($p<0.05$) compared with controls are marked with an asterisk.

pair-fed on a standard Larsen diet (Velaz) and allowed free access to water. The long-acting oestradiol benzoate preparation Agofollin Depot (Czecho-Slovakia) was administered s. c. - in doses of 1 mg per rat once every 5 days (experiment A), of 5 mg/kg body weight once every 5 days (experiment B) and 5 mg/kg body weight twice a week (experiment C). In the other groups, a corresponding amount of saline was administered, likewise subcutaneously. Norethisterone (Czechoslovakia) was added to the food in the concentrations given with the individual experiments and groups (actual consumption is given in the results). Both preparations were administered for four weeks before the experiment.

The local blood flow was determined by means of microspheres labelled with radioactive strontium ^{85}Sr (Kapitola *et al.* 1987,Rudolph and Heymann 1967). Ten to twelve minutes before injecting the microspheres, the rats were anaesthetized with pentobarbital in a dose of 60 mg per kg body weight. After the i.v. injection of heparin, a catheter was introduced into the right femoral artery and attached to a Type 304 peristaltic pump (Poland) functioning as an artificial organ for the determination of cardiac output. Another catheter, attached to a LMP 160 pressure transducer, a LDP 186 blood pressure recorder and LKM 210 cardiomonitor (Tesla, Czechoslovakia), was introduced into the right carotid artery and - with constant control of the pressure curve - into the left ventricle of the heart. The ^{85}Sr-microspheres (diameter 15 lm, in 10 % dextran solution, a 3M product USA), in a dose of approximately 18.5 kBq, i. e. 0.5 lCi, in 0.3 ml volume, were injected within 10 s; afterwards, the catheter was washed out with 0.3 ml physiological saline. About 1 min later the rat was sacrificed by decapitation, the organs and tissue samples were removed and the bones cleaned. All samples were weighed and measured, together with ^{85}Sr-microsphere standards, in a NA 1501 Gammaautomat (Tesla, Czechoslovakia). The ^{85}Sr-microsphere content was determined as the percentage of the dose in 1 g tissue and cardiac output and the local blood flow in the relevant tissues were computed from known formulas. The left tibia, the distal part of the left femur (for a distance of 7 mm) and the adrenals, uterus, ovaries and testes were dissected out.

After first weighing the tibia on a PRLT TW 2 torsion balance (Poland), under water and in the air, bone density was computed according to the principle of Archimedes. Tibial ash weight was determined after about 18 hours burning in a muffle furnace at 800 °C, by weighing the ash on a 2004 MP analytical balance (Sartorius, Switzerland).

The significance of differences was evaluated by an analysis of variance, using Duncan's test, the computation was done by Mrs. M. Dlouhá.

Results

Experiment A - females (Table 1). The blood flow in both the tibia and the distal femur fell significantly after oestradiol benzoate. Norethisterone was added to the food in a concentration of 0.002 % and with a mean consumption 0.5 mg/rat/day no changes were recorded in the local blood flow. Cardiac output was not affected by either of the two preparations.

Experiment B - females (Figure 1, Table 2). After oestradiol benzoate, the blood flow in the tibia and the distal femur fell. In the 0.002 % concentration, and with a mean consumption of 0.5 mg/rat/day,

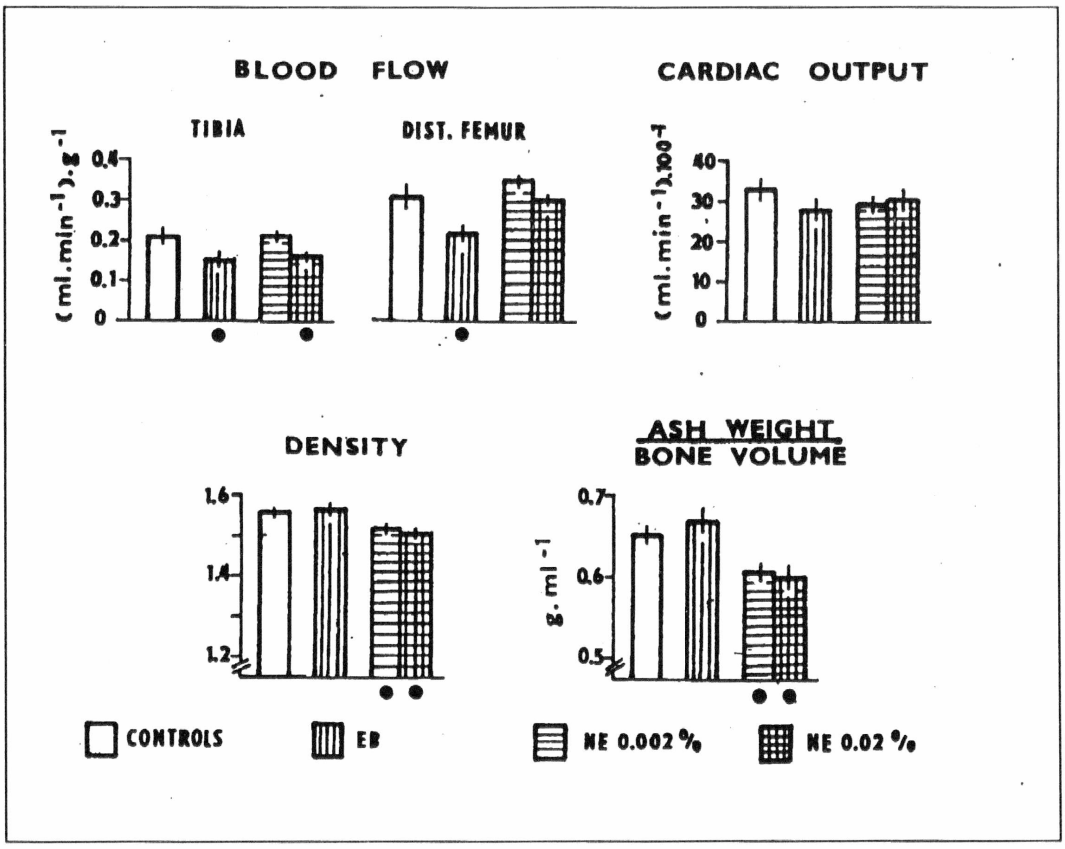

Fig. 1

Blood flow in the tibia and distal part of the femur, cardiac output and tibial mineralization indexes of female rats given oestradiol benzoate (long-acting preparation, 5 mg/kg s. c. every 5 days for 4 weeks) and norethisterone (0.002 % in the food for four weeks). Experiment B. Mean values ± standard errors of the means. Statistically significant differences ($p<0.05$) compared with the controls are marked with a black circle.

Table 2

Relative adrenal, uterine and ovarian weight in female rats given long-acting oestradiol benzoate (5 mg/kg s. c. once every 5 days) and norethisterone (0.002 % and 0.02 % in the food). Experiment B.

Group	Controls	OEB	NE 0.002 %	NE 0.02 %
Number of rats	10	9	10	9
Weight of rats (in g)	270±7	211±5*	226±5*	197±7*
Relative weights (in mg per 100 g):				
adrenals	5.2±1.4	29.5±2.8	27.3±1.5	30.0±6*
uterus	146.3±6.7	239.4±20.3*	164.0±10.6	235.2±26.2*
ovaries	39.4±2.3	56.0±4.0*	35.6±1.7	35.0±3.2

Means ± standard errors of the means.
Statistically significant differences ($p<0.05$) compared with controls are marked with an asterisk.

norethisterone produced no demonstrable changes, but in the 0.02 % concentration, and with a mean consumption of 4.3 mg/rat/day, it led to a significant decrease in the tibial blood flow. The cardiac output did not change. Bone density and ash weight per bone volume unit was nonsignificantly higher after oestradiol benzoate and significantly reduced after both doses of norethisterone. Relative adrenal weight was nonsignificantly higher after oestradiol benzoate and significantly raised after the addition of 0.02 %

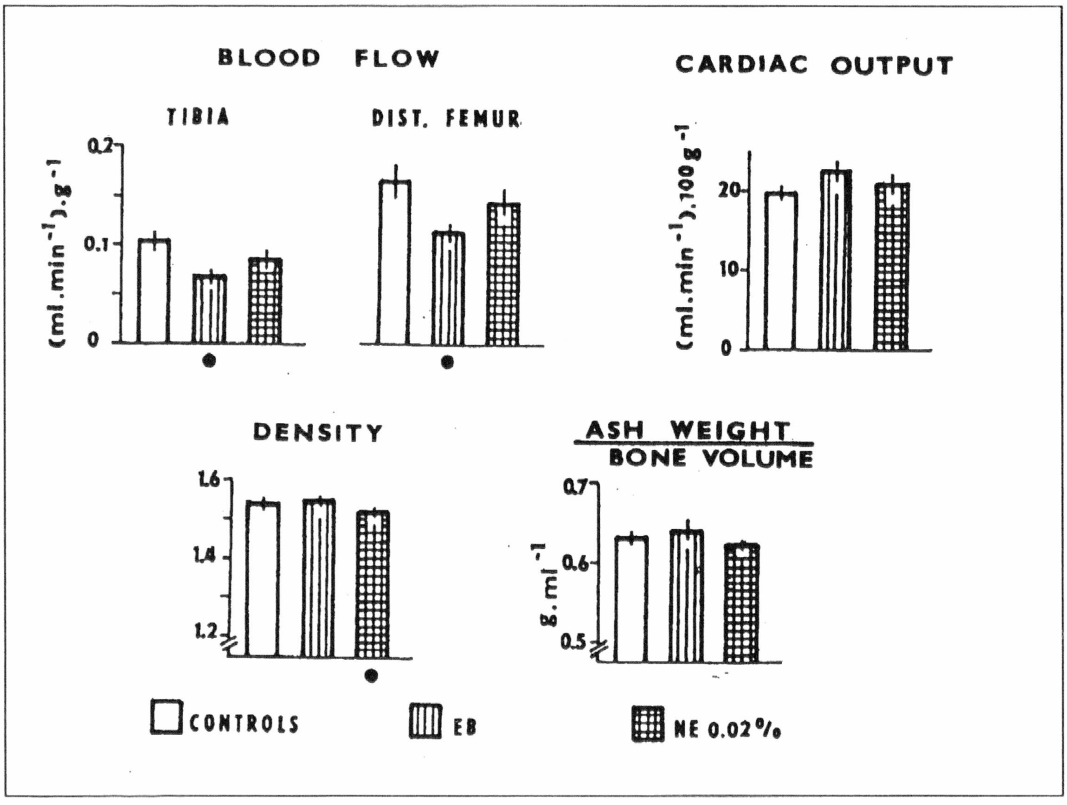

Fig. 2

Blood flow in the tibia and distal part of the femur, cardiac output and tibial mineralization indexes of male rats given oestradiol benzoate (long-acting preparation, 5 mg/kg s. c. twice a week for 4 weeks). Experiment C. Mean values ± standard errors of the means. Statistically significant differences ($p<0.05$) compared with the controls are marked with a black circle.

Table 3

Relative adrenal and testicular weight in male rats after administration of oestradiol benzoate (5 mg/kg s. c. twice a week) and norethisterone (0.02 % in the food). Experiment C.

Group	Controls	OEB	NE
Number of rats	10	9	8
Weight of rats (in g)	497 ± 14	382 ± 9*	401 ± 21*
Relative weights (in mg per 100 g):			
adrenals	13.2 ± 0.9	19.2 ± 0.6*	15.1 ± 1.1
testes	744.5 ± 31.6	283.3 ± 16.7*	564.7 ± 26.4*

Means ± standard errors of the means.
Statistically significant differences ($p<0.05$) compared with controls are marked with an asterisk.

norethisterone to the food. Relative uterine weight was significantly increased after both oestradiol benzoate and norethisterone in the concentration of 0.02 %. Relative ovarian weight rose significantly only after oestradiol benzoate.

Experiment C - males (Figure 2, Table 3). The blood flow in both bones was markedly reduced after oestradiol benzoate, but after a norethisterone concentration of 0.02 % in the food and mean consumption of 2.4 mg/rat/day the decrease was very slight (the lower norethisteron consumption was due to the lower food consumption). Cardiac output did not alter. Bone density and ash weight per bone volume unit were nonsignificantly greater after oestradiol benzoate. After norethisterone, bone density fell significantly, ash weight nonsignificantly. Relative adrenal weight was raised significantly after oestradiol benzoate and nonsignificantly after norethisterone,

while relative testicular weight fell significantly after both preparations.

Discussion

As regards the effect of oestradiol benzoate on the bone circulation, the results of the experiments described in this study always showed a significant decrease in the blood flow, as in a number of earlier experiments (Kapitola and Kubíčková 1990, Kapitola *et al.* in press).

The effect of norethisterone varies with the dose. When norethisterone was administered in the food in the 0.002 % concentration – which corresponds roughly to the dosage for oestradiol benzoate (according to usual clinical practice) – no local circulatory change can be demonstrated in the bones. With a dose one order higher, the bone blood flow displayed a tendency to fall, but the drop was significant in only one case (in the tibial blood flow in experiment B). In larger doses, the oestrogenic component of norethisterone may be manifested in its effect.

In principle, therefore, the effects of oestradiol benzoate and norethisterone on the rat bone blood flow differ in the similar way as their effects on indexes of osteoblastic activity in the clinical observation cited above (Štěpán *et al.* 1989, Johansen *et al.* 1990). By analogy, the difference in the effects of oestradiol and norethisterone on the blood flow could mean that the osteoblasts participate in the decrease in the blood flow after oestradiol. The results can thus be regarded as indirect evidence of the possible role of the osteoblasts in regulation of the bone blood flow.

In experiments B and C, tibial bone density and ash weight per bone volume unit were only nonsignificantly raised, after oestradiol, whereas the difference in the majority of earlier experiments were mostly significant. The results of these indexes after norethisterone were surprising: in every group there was a decrease - mostly significant and even after lower norethisterone concentrations in the food. This experimental finding in rats draws attention to the possibility that norethisterone may have a negative effect on bone mineralization and ought to be verified under other conditions.

When checking the general effects of oestradiol benzoate and norethisterone in the given doses and by given routes, we recorded the relative weights of the adrenals and genital organs. After oestradiol and larger doses of norethisterone, relative adrenal weight was raised, in part significantly. Relative uterine weight rose significantly after both estradiol and higher doses of norethisterone, while relative ovarian weight rose after oestradiol, but did not change after norethisterone. Relative testicular weight decreased after both preparations.

The above results are a further contribution to the still open question of the regulation of bone blood flow and its significance. Once again, they draw attention to the possible role of osteoblasts – a problem which will require further experimental work.

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