Physiol. Res. 44: 179 – 184, 1995

Inhibitory Effect of Hydrocortisone on the Blood Flow and ^{45}Ca and ^{3}H-Proline Incorporation into Bones of Female Rats

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Received February 9, 1995
Accepted March 27, 1995

Summary

We studied the effects of hydrocortisone as a possible regulatory factor of bone blood flow and metabolism. Local bone blood flow in the tibia, distal femur, lumbar vertebra and some soft tissues (using ^{85}Sr-microspheres), as well as ^{45}Ca and ^{3}H-proline incorporation into the tibia, bone density and ash weight per ml of the tibia were measured in sham-operated and oophorectomized female rats in which the influence of hydrocortisone administration (0.004 % diet for 5 weeks) was followed. Hydrocortisone markedly lowered ^{85}Sr-microsphere uptake and blood flow through the bones of non-castrated female rats as well as elevated circulatory values in oophorectomized rats. The changes were nearly identical in the three bone samples measured; among the soft tissues only the kidneys showed a less pronounced decrease. Circulatory changes in the bones seem to be caused by local vascular reactions. Hydrocortisone also lowered the 24-hour incorporation of ^{45}Ca and ^{3}H-proline into the tibia of both non-castrated and oophorectomized females. In the tibia of oophorectomized rats, hydrocortisone normalized the decreased bone density and ash weight. The adrenocortical hormones are known to block eicosanoid synthesis by the inhibition of arachidonic acid production. It is possible, therefore, that local circulatory changes in the bones of rats, induced by hydrocortisone, are mediated by the changes in prostaglandin production.

Key words

Bone blood flow – Hydrocortisone – Prostaglandins – Rat

Introduction

In a preliminary communication, (Kapitola *et al.* 1992) demonstrated a marked reduction of bone blood flow in intact female rats as well as elevated blood flow in oophorectomized rats induced by the administration of hydrocortisone. This finding could be of some interest with respect to the still obscure question of bone blood flow regulations and to other known actions of corticoid hormones on bones. We performed, therefore, further experiments with the aim to confirm our initial results, to compare the circulatory changes in bones and in some other tissues and also to estimate the 24-hour incorporation of ^{45}Ca and ^{3}H-proline into the tibia.

Material and Methods

Three experiments were performed on 131 70-day-old female rats (Institute of Pharmacy, Prague, Czech Republic) fed a standard laboratory diet (Bergman, Jesenice, Czech Republic) and drinking water *ad libitum*. The experiments were carried out according to the following procedure. Group 1: controls (sham operation), Group 2: oophorectomy (OOX), Group 3: hydrocortisone (sham operation), Group 4: OOX + hydrocortisone. Oophorectomy was performed by the dorsal approach five weeks before the actual experiment. Hydrocortisone (Léčiva, Prague, Czech Republic) in a 0.004 % concentration was added to the food for five weeks preceding the experiment.

The local blood flow was determined from the uptake of microspheres labelled with radioactive strontium (^{85}Sr) (Rudolph and Heymann 1967, Kapitola *et al.* 1987). The rats were anaesthetized with pentobarbital and were given an i. v. injection of heparin. A catheter was introduced into the right femoral artery and connected to a Type 304 peristaltic pump (Poland), which acted as an artificial organ for the determination of cardiac output. Another catheter,

which was connected to LMP 160 pressure transducer with LDP 186 blood pressure recorder and LKM 210 cardiomonitor (Tesla, Czech Republic), was introduced *via* the right carotid artery into the left heart ventricle (under careful control of the pressure curve). Through this tubing we injected a dose of approximately 18.5 MBq, i. e. 0.5 μCi, ^{85}Sr-microspheres (diameter 15 μm, NEN, USA) and then rinsed it with saline. The rat was decapitated after about one minute. The following organs and samples were removed: left tibia, an approximately 7 mm segment of the distal end of the left femur, the first lumbar vertebra (all bone samples were cleaned of soft tissues), kidneys, one lobe of the liver, terminal 5–7 cm segment of the colon, m. gastrocnemius and a sample of the skin from the ventral part of the body. The samples were weighed on a PRLT TW2 torsion balance (Poland) and measured together with ^{85}Sr-microsphere standards in a Gamma automat NA 3601 (Tesla, Czech Republic). The uptake of ^{85}Sr-microspheres was expressed as the percentage of the dose per 1 g of tissue (this value is also a relative indicator of local circulatory changes, independent of simultaneous changes in cardiac output). The local blood flow and cardiac output were computed according to generally employed formulas (Kapitola *et al.* 1987).

The 24-hour incorporation of ^{45}Ca and ^{3}H-proline, as an indicator of mineralization and of the formation of the organic matrix, was determined after Globus *et al.* (1986). 7.4 kBq, i.e. 0.2 μCi ^{45}Ca, in the form of $CaCl_2$ (Poland) and 185 kBq, i. e. 5 μCi, ^{3}H-proline (Amersham, England) per 100 g body weight were injected i. p. in a single dose. The next day, the rats were sacrificed, their left tibia was dissected out, cleaned and dissolved in concentrated HCl. Some of the diluted material was measured in an Insta-Gel liquid scintillator (Packard, USA) on a 1219 Rackbeta Liquid Scintillation Counter (LKB, Finland). The results are given as the number of dpm per mg osseous tissue. Bone density was computed on the basis of the principle of Archimedes, after weighing the tibia on a PRLT TW2 torsion balance under water and in the air. Ash weight was determined after incinerating the bone for about 18 h in a muffle furnace at 800 °C, by weighing the ash on a 2004 MP balance (Sartorius, Germany). The results are given in mg of ash per ml bone volume.

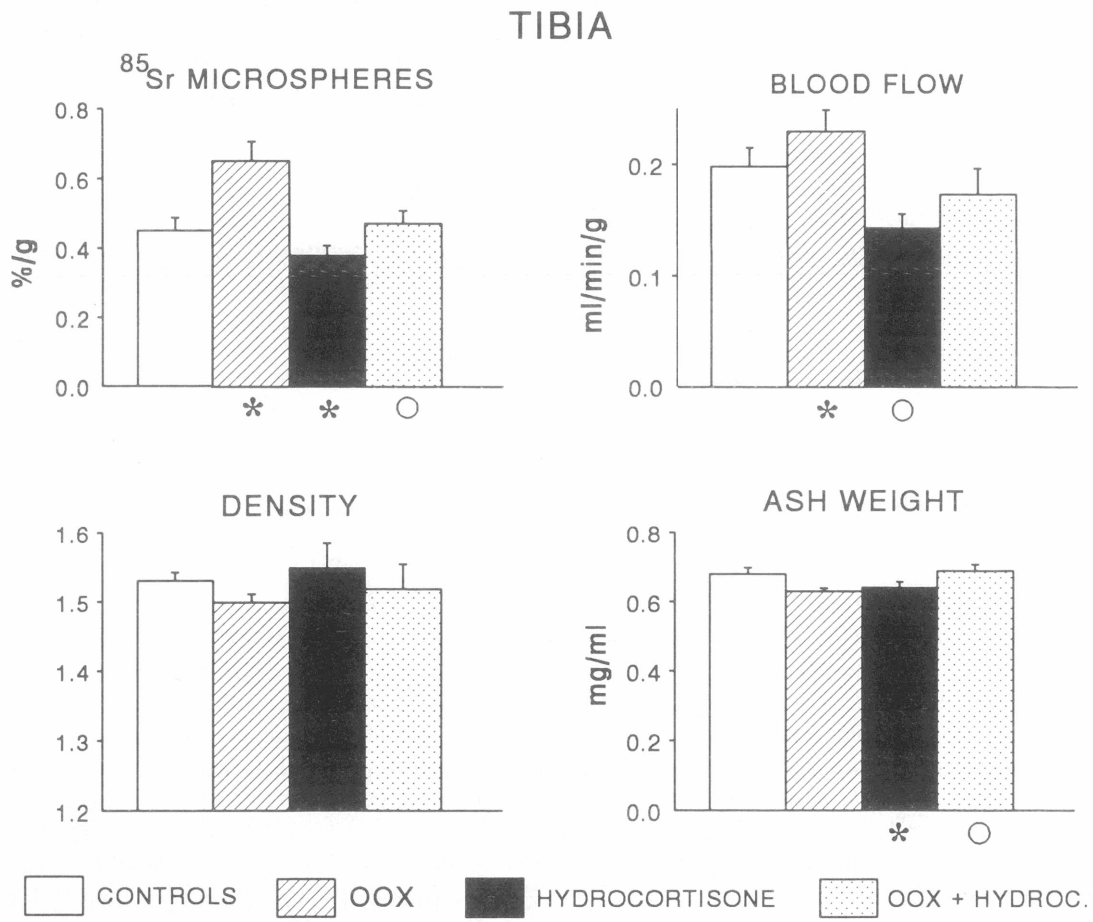

Fig. 1
^{85}Sr-microsphere uptake, local blood flow, bone density and ash weight of the tibia in female rats – sham-operated controls and oophorectomized rats (OOX): effects of hydrocortisone administration (0.004 % in the food for 5 weeks). Means ± S.E.M. Statistically significant differences ($p<0.05$): * compared with controls, ° compared with OOX group.

Statistics

To assess the significance of group differences we used the one-way analysis of variance. The appropriateness of the ANOVA model was checked by investigating whether the data within each group appeared to be normally distributed and whether the population variance was the same in each group. Standardized skewness and kurtosis was used for testing the assumption of normality and the Bartlett test was employed to test the equality of variances. The non-parametric Kruskal-Wallis test was used for parameters where one of these assumptions was not fulfilled. As a multiple comparison test we used the Scheffe or LSD test.

Results

Experiment A verified the changes of local blood flow in the tibia of female rats induced by hydrocortisone (Fig. 1, Table 1). ^{85}Sr-microsphere uptake and blood flow in the tibia decreased after hydrocortisone in non-castrated rats (as compared with the controls) as well as in the OOX animals (as compared with the OOX group). Cardiac output per 100 g body weight was reduced significantly in OOX rats, but was not modified by the administration of hydrocortisone.

Experiment B investigated local blood circulation in the tibia, distal femur, vertebra and in some soft tissues (Tables 2 and 3). ^{85}Sr-microsphere uptake in bone samples was markedly lower in non-castrated females after hydrocortisone than in the controls (non-significant in the vertebra). Values in OOX females receiving hydrocortisone were significantly elevated in all bone samples. The decrease in blood flow values after hydrocortisone was more pronounced than that of the microsphere uptake because of a concomitant decrease in cardiac output. In soft tissue samples, there were no significant changes in the microsphere uptake values after hydrocortisone; some of the lowered values of blood flow through the kidneys, intestine and skin after hydrocortisone were due to decreased cardiac output. The decrease in cardiac output was significant in the OOX rats only when expressed per 100 g body weight. The heart rate did not change, blood pressure increased significantly only in the OOX + hydrocortisone group. The density and ash weight per ml tibia volume was lower in the OOX groups, the ash weight rose after hydrocortisone in OOX rats.

In experiment C, we examined 24-hour incorporation of ^{45}Ca and ^{3}H-proline in the tibia (Table 4). The incorporation of both indicators in the tibia tended to be higher in OOX females. After hydrocortisone it decreased in intact rats (significantly as compared with controls) as well as in OOX females (significantly against the OOX group).

Table 1
Body weight and cardiac output in female rats – sham-operated controls and oophorectomized rats (OOX) with or without hydrocortisone (0.004 % in the food for 5 weeks)

Group	Controls	OOX	Hydro-cortisone	OOX + hydro-cortisone
Number of rats	12	12	12	12
Body weight (g)	233±6	237±7	231±7	235±9
Cardiac output				
(ml/min)	48.7±3.6	47.8±2.6	41.5±2.8	41.0±4.2
(ml/min per 100 g)	21.8±1.5	17.4±1.1*	19.0±1.2	18.2±2.0

*Means ± S.E.M. *Significantly different from the controls ($p<0.05$)*

Table 2
Uptake of ^{85}Sr-microspheres in the bones and some soft tissues and mineral content in the tibia of female rats – sham-operated controls and oophorectomized rats (OOX): effects of hydrocortisone administration (0.004 % in the food for 5 weeks)

Group	Controls	OOX	Hydro-cortisone	OOX + hydro-cortisone
Number of rats	11	11	9	11
Body weight (g)	244 ± 2.2	274 ± 5*	231 ± 4	254 ± 4$^{+\#}$
^{85}Sr-microsphere uptake (%/g)				
Tibia	0.53 ± 0.03	0.64 ± 0.06	0.31 ± 0.06*	0.40 ± 0.04^{+}
Distal femur	0.73 ± 0.04	0.87 ± 0.07	0.41 ± 0.05*	0.55 ± 0.05^{+}
Vertebra	0.69 ± 0.06	0.84 ± 0.05	0.49 ± 0.06	0.59 ± 0.05^{+}
Kidneys	12.89 ± 0.80	12.77 ± 1.05	10.14 ± 0.71	11.29 ± 1.07
Liver	0.18 ± 0.04	0.19 ± 0.03	0.22 ± 0.04	0.19 ± 0.03
Intestine	2.20 ± 0.26	1.96 ± 0.40	1.76 ± 0.20	1.81 ± 0.23
M. gastrocnemius	0.17 ± 0.02	0.18 ± 0.01	0.16 ± 0.02	0.19 ± 0.02
Skin	0.21 ± 0.03	0.21 ± 0.02	0.14 ± 0.02	0.21 ± 0.02
Bone density (tibia)	1.57 ± 0.01	1.54 ± 0.01*	1.58 ± 0.004	1.55 ± 0.01$^{\#}$
Ash weight (tibia) (mg/ml)	0.67 ± 0.01	0.63 ± 0.01*	0.68 ± 0.01	0.66 ± 0.01

*Means ± S.E.M. Significantly different ($p < 0.05$) from: * controls, $^{+}$ OOX, $^{\#}$ hydrocortisone*

Table 3
Blood flow in the bones and some soft tissues and circulatory values in female rats – (sham-operated controls) and oophorectomized rats (OOX): effects of hydrocortisone administration (0.004 % in the food for 5 weeks)

Group	Controls	OOX	Hydro-cortisone	OOX + hydro-cortisone
Cardiac output (ml/min)	52.0 ± 3.0	54.6 ± 5.6	39.3 ± 4.8	40.4 ± 5.4
(ml/min per 100 g)	20.5 ± 1.8	20.3 ± 2.4	17.2 ± 2.2	15.8 ± 2.0
Heart rate (beats per min)	375 ± 9	376 ± 12	391 ± 12	411 ± 9
Blood pressure (kPa)	15.3 ± 0.4	14.8 ± 0.4	14.7 ± 0.8	17.7 ± 0.5$^{*+\#}$
Blood flow (ml/min per g)				
Tibia	0.22 ± 0.01	0.24 ± 0.02	0.13 ± 0.02*	0.12 ± 0.01^{*+}
Distal femur	0.31 ± 0.02	0.34 ± 0.03	0.14 ± 0.01*	0.16 ± 0.01^{*+}
Vertebra	0.28 ± 0.02	0.33 ± 0.04	0.16 ± 0.02*	0.17 ± 0.01^{*+}
Kidneys	5.64 ± 0.68	5.15 ± 0.66	3.24 ± 0.43*	3.17 ± 0.21*
Liver	0.07 ± 0.01	0.11 ± 0.05	0.06 ± 0.01	0.06 ± 0.01
Intestine	0.94 ± 0.15	0.68 ± 0.09	0.58 ± 0.10	0.50 ± 0.05*
M. gastrocnemius	0.07 ± 0.01	0.07 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
Skin	0.09 ± 0.01	0.08 ± 0.01	0.05 ± 0.01*	0.06 ± 0.01

*Means ± S.E.M. Significantly different ($p < 0.05$) from: * controls, $^{+}$ OOX, $^{\#}$ hydrocortisone*

Table 4
24-hour incorporation of ^{45}Ca and ^{3}H-proline in the tibia of female rats – sham-operated controls and oophorectomized rats (OOX): effects of hydrocortisone administration (0.004% in the food for 5 weeks)

Group	Controls	OOX	Hydro-cortisone	OOX + hydro-cortisone
Number of rats	11	10	10	9
Body weight (g)	231 ± 3	261 ± 4*	211 ± 2*	236 ± 7$^{+\#}$
^{45}Ca incorporation	4.18 ± 0.16	4.32 ± .0.19	3.20 ± 0.14*	3.38 ± 0.25$^{+\#}$
^{3}H-proline incorporation	3.88 ± 0.18	4.53 ± 0.17	3.00 ± 0.14	3.22 ± 0.26^{+}

*Means ± S.E.M. Significantly different ($p < 0.05$) from: * controls, $^{+}$ OOX, $^{\#}$ hydrocortisone*

Discussion

The present experiments confirm the marked and reproducible inhibitory effect of hydrocortisone on bone blood flow. The effect is nearly identical in the three bone samples studied. Among the soft tissues studied, a less pronounced decrease of blood flow after hydrocortisone was found in the kidneys; the changes in the intestine and skin were of no importance. Thus, the fully expressed local circulatory action of hydrocortisone could be demonstrated in the bones only.

The inhibition of 24-hour incorporation of both ^{45}Ca an ^{3}H-proline was also very marked. This indicates (with a high probability) a functional importance of blood flow in the process of bone formation. The uptake of calcium in the tibia was suppressed by hydrocortisone while the bone mineral content did not fall. On the contrary, the decrease in the ash weight per bone volume in OOX rats became normalized after the administration of hydrocortisone. A similar situation, i. e. an increase in bone mass accompanied by inhibition of bone mineralization, was found after the administration of $1,25(OH)_2D_3$ vitamin (Wronski *et al.* 1986).

The main reason of this work was particularly the question of bone blood flow regulations. We know that the bone blood flow rises in castrated rats of both sexes (Schoutens *et al.* 1984, Kapitola *et al.* 1991) and declines after the administration of sex hormones in intact and castrated animals (Kapitola *et al.* 1990, Kapitola *et al.* 1995). The mechanism of these changes is, however, unknown. One possibility might be the mediation by prostaglandins, most probably by PGE_2. PGE_2 is produced in the bone tissue and plays an important role in the regulation of bone metabolism (Watrous and Andrews 1989). The production of PGE_2 by the rat parietal bones *in vitro* is lowered by oestradiol given *in vivo* and also by cortisol added *in vitro* (Feyen and Raisz 1987). Our previous results show that bone blood flow (including its rise after oophorectomy) may be suppressed by the administration of acetylosalicylic acid (Kapitola *et al.* 1994), possibly as a consequence of the inhibition of prostaglandin synthesis. Corticoid hormones are known to block the metabolic pathways of eicosanoids by inhibiting arachidonic acid production (Foegh *et al.* 1991). It is possible, therefore, that the lowering of bone blood flow after hydrocortisone may be brought about in the same way.

The inhibitory action of hydrocortisone on bone blood flow as well as on ^{45}Ca and ^{3}H-proline incorporation is not actually known. It must be mentioned that we are dealing with experimental conditions and with high doses of hydrocortisone. Nevertheless, the observed effects are impressive and convincing and must be taken into consideration.

Acknowledgement
This work was supported by grant No. 306/93/0851 of the Grant Agency of the Czech Republic.

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