Physiol. Res. 42: 415–418, 1993

Effect of Somatotropin on Uterine Oestrogen Receptor Concentration in Vitamin C-deficient Guinea-Pigs

J. BÁRTOVÁ, J. ŠKARDA

Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Prague

Received April 26, 1993
Accepted July 8, 1993

Summary

Somatotropin treatment does not stimulate body growth in guinea-pigs, whereas it is effective in stimulation of both cytosolic and nuclear uterine ooestradiol receptor concentration in the animals fed optimum amounts of ascorbic acid. To determine whether this effect of somatotropin is ascorbic acid-dependent, guinea-pigs of 9 weeks of age with marginal vitamin C deficiency were treated with either recombinant bovine somatotropin (bST; 0.5 mg per animal) or vehicle for 10 days. The amount of available cytosolic ooestradiol receptor per unit of uterine weight, the DNA content, or in whole uteri was increased in somatotropin-treated animals (3.3 to 5.9-fold) compared to controls. However, the nuclear uterine oestradiol receptor concentration was not increased. The dissociation constant values were significantly higher in the cytosol (control: 2.79, bST-treated: 2.66) than in the nuclear fraction (control: 1.76, bST-treated: 1.80) and did not differ between control and bST treated animals. The results of this investigation demonstrate that guinea-pigs with marginal vitamin C deficiency provide a suitable model for studying the effect of vitamin C on somatotropin action. The possible synergistic action of ascorbic acid on uterine action of somatotropin is discussed.

Key words

Somatotropin – Vitamin C deficiency – Oestrogen receptor – Uterus – Guinea-pig

Introduction

The metabolic effects of ascorbic acid are not entirely known. However, the number of biological reactions in which ascorbic acid has been shown to play a role is increasing rapidly. It is essential as a coenzyme in certain oxidative-reductive processes, and it is also essential in hydroxylating systems. The activities of amidases, proteases, glycosidases, peroxidases, esterases, arginase, papain, liver esterase, catalase and catepsin are increased while the activities of urease, β-amylase and lipase are decreased. A synergistic relationship to other vitamins was proved for vitamin A, E, K, B_6, B_{12}, folic acid and pantothenic acid. The relationships to steroid hormones, catecholamines, serotonin, thyroxine, FSH, LH, ACTH and to the synergistic action of ascorbic acid with somatotropin in growth regulation were demonstrated (Tsai and Vaughan 1972, Kutsky 1973, Robinson *et al.* 1973, Tsai *et al.* 1973, Brander 1982, Ginter 1989). The synergistic relationship of ascorbic acid to the growth-promoting actions of somatotropin can be expected in primates but not in guinea-pigs, as somatotropin does not act as a body growth-promoting hormone in this species (Mitchell *et al.* 1954). However, the relationship between the somatotropin action and ascorbic acid can be studied on the basis of our previous experiments that provided evidence for an unusual function of somatotropin in the guinea-pig uterine tissue. Bovine somatotropin (bST) increased the concentration of both cytosolic and nuclear ooestradiol receptors in the uterine tissue but not in the mammary glands (Bezecný *et al.* 1992). The aim of the present study was to measure the effect of bST on uterine ooestradiol receptor levels in vitamin C-deficient guinea-pigs.

Methods

Animals and hormone treatments

Mixed strains of guinea-pigs were obtained from a commercial breeder (Velaz, Prague, Czech Republic). Animals were housed in cages (five animals per cage) in a room with controlled temperature (23±2 °C) under 12 h light and 12 h darkness. The

animals were allowed to adapt to these housing conditions and to a marginal vitamin C-deficient diet for 3 to 4 weeks before the experiment. The following model of marginal vitamin C deficiency was used. The diet for guinea-pigs was gradually (during 7 days) changed to Lunde's diet (Hanke 1943) modified according to Ginther (1964) (Table 1). The guinea-pigs were kept on the vitamin C-free Lunde's diet for 2 weeks. This reduced the ascorbate levels in animals' tissues, although vitamin C deficiency was not yet clinically manifest. Then a maintenance dose of ascorbic acid (0.5 mg/kg/day dissolved in 1 ml of water) was applied through a stomach tube. Drinking water was available *ad libitum*. The body weight gains and behaviour of marginally deficient guinea-pigs were the same as those of age-matched animals but fed the guinea-pig pelleted diet (MOK; Velaz, Lysá nad Labem, Czech Republic) supplemented with fresh green grass (Bezecný *et al.* 1992).

Table 1
Composition of vitamin C-deficient diet for guinea-pigs

Ingredient	Amount
Oat flakes	2000 g
Bran	800 g
Milk powder	600 g
Butter	400 g
Fish oil	5 ml
Vitamin B supplement[1]	0.55 g
NaCl	400 g
Mineral supplement[2]	20 g

[1]) *Vitamin B supplement contained thiamine hydrochloride (40 mg), riboflavin (40 mg), pyridoxine hydrochloride (20 mg), pantothenic acid (calcium salt, 60 mg), nicotinamide (400 mg).*

[2]) *Mineral supplement contained* $MgSO_4 . 7H_2O$ *(27.8 g),* $CaCO_3$ *(52.6 g),* Na_2HPO_4 *(16.65 g),* $K_2CO_3 . 1.5H_2O$ *(55.9 g),* $FeCl_3$ *(1.64 g),* $MnSO_4 . 5H_2O$ *(30 mg), KI (7.8 mg), NaF (9.7 mg),* $KAl(SO_4)_2 . 12H_2O$ *(9.4 mg), citric acid (43.3 g).*

Female guinea-pigs were treated s.c. daily for 10 days, beginning at 9 weeks of age, with vehicle (control; 5 animals) or recombinant bovine somatotropin (bST; 5 animals, 0.5 mg per animal). The hormone was supplied by Eli Lilly, Indianapolis, IN, U.S.A. The animals were killed by decapitation 24 h after the 10th hormone or vehicle injection.

Preparation of tissue cytosolic and nuclear fractions

Uterine tissue was homogenized in liquid nitrogen and the homogenate was diluted with buffer: Tris-HCl (20 mmol/l), EDTA (2 mmol/l), dithiothreitol (0.05 mmol/l; Calbiochem, Luzerne, Switzerland), sodium molybdate (10 mmol/l), gelatin (0.1 % w/v), glycerol (10 % v/v), pH 7.4. The homogenate was centrifuged at 100 000 x *g* at 4 °C for 60 min and the supernatant (cytosol) was removed. The pellet was washed three times by vigorous resuspension in buffer and centrifuged at 1000 x *g* for 10 min at 4 °C. The final pellet (nuclear fraction) was resuspended in the same volume of buffer as the original homogenate.

Steroids

(^{3}H)ooestradiol (Amersham International, Amersham, U.K.; specific activity 1.89 TBq/mmol) was purified by Sephadex LH-20 (Pharmacia, Uppsala, Sweden) column chromatography (70x10 mm) using a benzene-methanol (9:1, v/v) solvent system. The ooestradiol fraction was dried under N_2 and then dissolved in methanol and stored at −20 °C until use. Radioactive ooestradiol was diluted with a buffer to the appropriate activity (approx. 10^6 d.p.m./ml) from which serial dilutions (0.1–6 nmol/l) were prepared for the determination of displaceable binding. Non-radioactive ooestradiol and diethylstilbestrol (DES) were obtained from Koch-Light Laboratories, Colnbrook, U.K.

Determination of (^{3}H)ooestradiol binding

(^{3}H)ooestradiol solutions, with or without DES (100 µl), were introduced into plastic tubes and the cytosolic and nuclear fractions (100 µl) were added and incubated at 4 °C for 18–20 h. After incubation, unbound steroid was removed using dextran-coated charcoal for the cytosolic fraction and buffer washings of nuclear suspensions as has been described previously (Bezecný *et al.* 1992). The binding data were analyzed according to Scatchard (1949) and the dissociation constant (K_d) and the number of receptor binding sites were calculated. All the data presented on specific binding were obtained following subtraction of the non-specific binding from total binding, as described by Chamnes and McGuire (1975). Non-specific binding was calculated from samples containing a 100-fold excess of non-labelled DES.

Protein, RNA and DNA determination

The tissue and cytosolic protein contents were determined by the method of Lowry *et al.* (1951) using bovine serum albumin (fraction V; Armour, Kankakee, IL, U.S.A.) as the standard. DNA concentrations were estimated with indole by a modified method as described by Urbanová *et al.* (1985).

Statistical analysis

Values are expressed as means ± S.E.M. Statistical differences between treated and control animals were estimated by Student's t-test. Values were considered to be significantly different when $P < 0.05$.

Results

The dose of ascorbic acid (0.5 mg/kg/day) maintained the relative body weight gains at a level of 22 to 28 % which is the range of growth found in animals of the same age but fed the diet supplemented with vitamin C by feeding fresh grass and cabbage (Bezecný *et al.* 1992). However, the low maintenance dose of ascorbic acid did not prevent the incidence of subcutaneous haemorrhage at the site of injection in both control and bST-treated animals.

Table 2
Effect of bovine somatotropin (bST) on ooestradiol receptor levels in the uterine tissue from female guinea-pigs with vitamin C deficiency. The guinea-pigs were treated s.c. daily for 10 days, beginning at 9 weeks of age with vehicle (control) or recombinant bST (0.5 mg per animal) and killed 24 h after the 10th injection.

	Control	bST
Uterine cytosol oestradiol receptor		
(fmol/g tissue)	2478 ± 163	4693 ± 310
(fmol/mg DNA)	176 ± 104	654 ± 104
(fmol/uterus)	2448 ± 263	8076 ± 1110
Uterine nuclear oestradiol receptor		
(fmol/g tissue)	2488 ± 202	2358 ± 165
(fmol/mg DNA)	173 ± 100	105 ± 16
(fmol/uterus)	2412 ± 178	1288 ± 175

Values are means ± S.E.M. from five animals.
$P < 0.01$ when compared to controls.

Table 2 shows that the concentration of available uterine cytosolic ooestradiol receptor per unit of uterine weight (fmol/g tissue) or DNA content (fmol/mg DNA) or per whole uterus (fmol/uterus) was increased in bST treated animals (3.3 to 5.9-fold) when compared with the controls. The nuclear ooestradiol receptor concentration was not affected by treatment when expressed per gram tissue, but was lowered when expressed per mg DNA or per uterus. The K_d values were higher in the cytosol (control: 2.79 ± 0.04 pmol/l; bST-treated: 2.66 ± 0.09 pmol/l) than in the nuclear fraction (control: 1.76 ± 0.15 pmol/l; bST treated : 1.80 ± 0.16 pmol/l) but did not differ between control and bST-treated animals.

Discussion

Scurvy is a metabolically poorly defined state, complicated by food refusal, drop in body weight, haemorrhage, etc. In general, multiple actions of vitamin C deficiency can be divided into those which are specific for only ascorbic acid and those that are nonspecific, which can be substituted by other compounds. Thus, acutely scorbutic guinea-pigs are not a suitable model for studying the specific reactions of ascorbic acid deficiency. The model of vitamin C deficiency recommended by Ginter (1978), and slightly modified in the present experiments by decreasing the maintenance dose of ascorbic acid from 1 mg to 0.5 mg/kg/day has far greater value for biochemical research. This is because a low ascorbic acid supplement can maintain the weight gains of young guinea-pigs at the level which occurs in animals of the same age but fed a diet rich in vitamin C (Bezecný *et al.* 1992). The incidence of subcutaneous haemorhages at the site of placebo or bST injections suggests that the dose of ascorbic acid was too low to prevent haemorhage in the wounded sites.

Responses of cytosolic ooestradiol receptor (per unit of uterine weight, per DNA content or in whole uteri) to bST treatment were increased in guinea-pigs with marginal vitamin C deficiency 3.3 to 5.9 times while in animals fed the optimum dose of ascorbic acid were increased 7.3 to 14.0 times when compared with the controls. The nuclear uterine ooestradiol receptor concentration was not affected by bST treatment in guinea-pigs with marginal vitamin C deficiency but increased by 3.3 to 6.7 times in animals fed the optimum dose of ascorbic acid (Bezecný *et al.* 1992). The reason why somatotropin treatment significantly increased the concentration of cytosolic but not nuclear oestrogen receptors is not known. According to this new concept, the oestrogen receptor is a nuclear receptor protein inactivated or bound to some nuclear constituent. The only time, the receptor is assumed to be in a soluble state is after its synthesis and during its transit into the nucleus (Gorski *et al.* 1984). Perhaps the transit of the receptor to the nucleus is impaired and/or the association of the unoccupied receptor in the nucleus is more readily disturbed during mechanical disruption of cells and cell fractionation in the uterine tissue of vitamin C-deficient guinea pigs.

The K_d values did not differ between control and bST- treated vitamin C-deficient animals. This suggests that uterine oestradiol receptors are regulated by bST through changes in the number of binding sites rather than by alteration of their binding affinity, similarly as in normal guinea-pigs (Bezecný *et al.* 1992).

The uterine action of somatotropin may involve interactions with putative somatotropin receptors in the endometrial epithelium and stroma and with somatotropin receptors in the myometrium. Somatotropin may also stimulate uterine production of insulin-like growth factor I (IGF-I) which, in turn enhances ooestradiol receptor formation by a paracrine and/or autocrine mechanism. Uterine actions of IGF-I produced in response to somatotropin treatment in the

liver and kidneys cannot be excluded (Phillips and Vassilopoulou-Sellin 1980). This can be supported by the fact that DNA synthesis in guinea-pig mammary explants was highly stimulated by IGF-I (Škarda, unpublished results).

In most species, somatotropin enhances the body growth of young animals and has a wide variety of metabolic actions in adults. In guinea-pigs, however, the administration of somatotropin fails to promote somatic growth in either intact or hypophysectomized animals, even though their pituitary extract contains somatotropin that can restore growth of hypophysectomized rats (Mitchell *et al.* 1954, Fairhall *et al.* 1990). The absence of the body growth stimulating effect of somatotropin (Bezecný *et al.* 1992) suggests that growth of any cell type is not directly stimulated by somatotropin in the guinea-pig.

The induction of uterine ostradiol receptors by somatotropin in guinea-pigs with marginal vitamin C deficiency provides a suitable model for studying the effect of vitamin C on somatotropin action, independently of its growth-promoting activity.

Acknowledgements

We would like to acknowledge the excellent technical assistance of Ing. I. Bezecný. We are also grateful to Dr. H. Mader and Dr. P. Kubasa from Eli Lilly and Elanco Vienna, Austria for the gift of recombinant bovine somatotropin.

References

BEZECNÝ I., BÁRTOVÁ J., ŠKARDA J.: Growth hormone treatment increases oestrogen receptor concentration in the guinea-pig uterus. *J. Endocrinol.* **134:** 5–9, 1992.

BRANDER G. C.: The vitamins. In: *Veterinary Applied Pharmacology and Therapeutics*. G.C. BRANDER, D.M. PUGH, R.J. BYWATER (eds), Bailliére Tindall, London, 1982, pp. 242–264.

CHAMNESS G.C., McGUIRE W.L.: Scatchard plots: common errors in correction and interpretation. *Steroids* **26:** 538–542, 1975.

FAIRHALL K.M., GABRIELSSON B.G., ROBINSON I.C.A.F.: Effect of food withdrawal and insulin on growth hormone secretion in the guinea pig. *Endocrinology* **127:** 716–723, 1990.

GINTER E.: Methodical comments concerning experimental vitamin C deficiency in guinea-pigs. (in Slovak), *Biológia* (Bratislava) **19:** 180–185, 1964.

GINTER E.: Marginal C deficiency, lipid metabolism and atherogenesis. *Adv. Lipid Res.* **16:** 157–220, 1978.

GINTER E.: Disorders of cholesterol metabolism in marginal vitamin C deficiency and the problem of optimum ascorbic acid intake. In: *Nutrition in the Prevention of Disease.* J.C. SOMOGYI, S. HEJDA (eds), Vol. 44, Karger, Basel, 1989, pp. 85–93.

GORSKI J., WELSHOMS W., SAKAI D.: Remodelling the estrogen receptor model. *Mol. Cell. Endocrinol.* **36:** 11–15, 1984.

HANKE H.: *Vitamine und Chirurgie*. G. Thieme, Leipzig, 1943.

KUTSKY R.J.: *Handbook of Vitamins and Hormones*. Van Norstrand Reinhold Co., New York, 1973.

LOWRY O.H., ROSENBROUGH N.J., FARR A.L., RANDALL R.J.: Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275, 1951.

MITCHELL M.L., GUILLEMIN R., SELYE H.: The effect of somatotropic hormone on the growth of normal and hypophysectomized guinea-pigs. *Endocrinology* **54:** 111–114, 1954.

PHILLIPS L.S., VASSILOPOULOU-SELLIN R.: Somatomedins. *New Engl. J. Med.* **302:** 371–380, 1980.

ROBINSON A.B., IRVING K., McCREA M.: Acceleration of the rate of deamination of Gly Arg Con Arg Gly and of human transferin by addition of L-ascorbic acid. *Proc. Natl. Acad. Sci. U.S.A.* **70:** 2122–2123, 1973.

SCATCHARD G.: The attraction of proteins for small molecules and ions. *Ann. N. Y. Acad. Sci.* **51:** 660–672, 1949.

TSAI S.C., VAUGHAN M.: Inactivation of hormone-sensitive lipase in extracts of adipose tissue. *J. Biol. Chem.* **247:** 6253–6258, 1972.

TSAI T.S., FALES H.M., VAUGHAN M.: Inactivation of hormone sensitive lipase from adipose tissue with adenosine triphosphate, magnesium and ascorbic acid. *J. Biol. Chem.* **248:** 5278–5291, 1973.

URBANOVÁ E., ŠEDINOVÁ V., ŠKARDA J., ŠKARDOVÁ O.,JUKL A.: The use of Synpor membrane filters for separation and determination of the number of somatic cells in milk of dairy cows by the DNA filter method. (in Czech), *Vet. Med. (Prague)* **30:** 409–418, 1985.

Reprint Requests

Dr J. Škarda, Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, 104 00 Prague 10, Uhříněves, Czech Republic.