Estrogen and Cortisol Receptors in Bone Cells

HORMONE RECEPTORS IN BONE:

AN EVALUATION OF THE UPTAKE OF ESTROGEN AND CORTISOL INTO BONE CELLS

by

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DEDICATED

to my wife, Rickie

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ABSTRACT

The uptakes of the radioactive hormones, ^{3}H-estradiol-17β and ^{3}H-cortisol, in bone cells were evaluated. Nuclear and cytoplasmic fractions were prepared from pulverized bone. Incubations were done in ^{3}H-estradiol-17β, ^{3}H-estradiol-17β plus tenfold amount of unlabelled estradiol, ^{3}H-cortisol, and ^{3}H-cortisol plus tenfold unlabelled cortisol. There was no difference in the uptake of ^{3}H-estradiol-17β alone or the ^{3}H-estradiol-17β plus unlabelled estradiol, leading to the conclusion that there is no specific binding of estrogens in bone cells. Cortisol uptake studies initially showed competitive uptake, but this uptake was later proven to be due to Transcortin, a plasma corticosteroid binding globulin. After dexamethasone uptake studies, a specific cellular binding glucocorticoid, no uptake was seen - signifying the absence of a cytoplasmic binding site for cortisol in bone. The lack of specific binding suggests absence of estrogen and cortisol receptor molecules in bone cells.

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RECEPTEURES HORMONOUX DANS L'OS:

Etude sur la Captation de L'estrogène et du Cortisol par la Cellule Osseuse

RESUME

La captation des hormones radioactives estradiol-^{3}H-17β et cortisol-^{3}H par la cellule osseuse a fait l'objet de cette étude. Des fractions nucléaires et cytoplasmiques ont été préparées à partir d'os pulvérisé. Quatre genres d'incubations ont été utilisés: 1) dans l'estradiol-^{3}H-17β, 2) dand l'estradiol-^{3}H-17β avec dix fois plus d'estradiol, 3) dans le cortisol-^{3}H, et 4) dans le cortisol-^{3}H avec dix fois plus de cortisol. On n'a constaté aucune différence dans la captation de l'estradiol-^{3}H-17β, seul, ou dans la captation de l'estradiol-^{3}H-17β avec l'estradiol. Cette observation nous indique l'absence de liens spécifiques des estrogènes dans la cellule osseuse. Les études sur la capatation du cortisol ont montré initialement une captation compétitive qu'on a pu par la suite attribuer à la transcortine, une globuline plasmique de lien pour les corticostéroides. Après des études de captation du dexamethasone, glucocorticoide de lien cellulaire spécifique, dans lesquelles aucune captation n'a été perçu, il apparait qu'un site de lien spécifique cytoplasmique pour le cortisol dans l'os n'existe pas. Le manque de lien spécifique, suggère l'absence dans la cellule osseuse, de récepteures moléculaires pour l'estrogène et le cortisol.

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INTRODUCTION

Effects of Estrogen on Bone

Kyes and Potter (1) showed that estrogen stimulated formation of deposits of spongy bone in the endosteum of preovulatory female pigeons. These deposits functioned to store calcium for later calcification of the eggshell. Estrogen affects intramedullary bone formation (2) in birds by inducing reticular cells to change into osteoblasts, which in turn change into osteocytes. Estrogen prevents reabsorption of endochondral spongy bone in growing rats (3) resulting in an elongated core of unreabsorbed bone filling several millimeters of the marrow cavity. Strontium-85 retention (4) was enhanced in estrogen treated mature female rats. An etiologic factor in development of osteoporosis may be a deficiency of gonadal hormones (5) and treatment with estrogen has caused bone resorption to return to a normal level in osteoporotic patients (6). Estrogen administration to rats with immobilized limbs (7) prevented the loss of calcium, bone rarefaction, and cortical thinning which are usually seen in immobilization osteoporosis. Changes induced in adult rats by administration of estrogen (8): a) different concentrations of lipid constituents in bone; b) increased rate of glucose utilization; c) decreased rate of epiphyseal mucopolysaccharide synthesis; and d) increased differentiation

of reticular cells into osteoblasts. Estrogen inhibits parathyroid hormone induced release of calcium in five day old mouse calvaria (9). Parathyroid hormone induced changes are greater in ovarectomized rats (10). In rat bone incubated in Krebs-Ringer bicarbonate buffer containing ^{3}H-6,7-estradiol-17β, the radioactivity in the bone increased in proportion to the length of incubation (11). The following study was carried out to determine whether estrogen is specifically bound by bone cells.

Effects of Cortisol on Bone

Administration of adrenal cortical steroids results in a varying picture in different species, ranging from gross rarefaction in the rabbit (12), to a metaphyseal sclerosis of rat bone (13, 14, 15). Prolonged cortisone therapy and Cushing's disease in man manifest as osteoporosis, the mechanism being increased resorption of bone and inhibition of bone formation (12, 16). Rats fed a calcium deficient diet sufficient to stimulate resorption also developed osteoporosis (12-15). The mechanism of cortisone induced osteoporosis is: a) Negative calcium balance (secondary to increased renal excretion of calcium and cortisone induced antagonism of vitamin D dependant intestinal absorption of calcium) stimulating hyperactivity of the parathyroid glands, which cause parathyroid hormone induced bone resorption; b) Anti-anabolic action which

decreases the rate and extent of osteoid formation so that there is an increased surface of calcified tissue available for bone resorption (12). Dexamethasone inhibits the parathyroid hormone induced release of calcium from embryonic rat bone tissue culture (17), but the osteoporotic effect of parathyroid hormone in calcium deficiency is increased further with cortisone administration (12), suggesting different direct and systemic effects on bone. This experiment explores the uptake of cortisol in bone cells in postulating whether corticosteroids have a direct mechanism of action on bone.

Estrogen Binding in Tissues

Jensen (18) in 1960 showed uterus and vagina to be target organs for estrogen in that they continue to incorporate estrogen, retain the steroid for a long time, and the tissue estrogen concentration is much greater than that of blood. In contrast, blood, kidney, muscle, and liver are non-target organs and reach their maximum estrogen content early, lose the steroid rapidly afterward, and the rapid loss of steroid parallels the rapid drop in blood steroid content. A model of estrogen receptors in target tissues based on subsequent experiments (19-28) explains the steps in steroid-cellular interaction. Estrogen in the blood and extracellular fluid comes into intimate contact with the target cell wall.

Estrogen migrates into the cell and interacts with a cytoplasmic protein to form an estrogen-receptor complex, an 8S complex composed of two 4S subunits and forms spontaneously at high or low temperatures. This cytoplasmic complex enters the nucleus where further changes occur that result in the appearance of estrogen bound to a 5S protein. The 5S complex is not formed spontaneously by adding estrogen to nuclei, but is formed from 8S complex by a process which consumes 8S receptor and is retarded at low temperatures. It is the 5S complex that retains the hormone and is responsible for chromatin interaction initiating cellular changes.

Cortisol Binding in Tissues

Studies on the mechanism of cortisol binding have been done in hepatoma tissue culture cells (29), liver (30), and thymus (31). Gardner and Tomkins (29) demonstrated a cytoplasmic, protein macromolecule which binds cortisol. Beato et al. (30) noted a 4S glycoprotein fraction in the cytosol which binds cortisol at high or low temperatures and is involved in the transport of hormone into the cell nucleus and in the binding to 9S nuclear macromolecules causing increased template capacity of the chromatin for RNA synthesis. Wira and Munck (31) isolated a cortisol-receptor complex in the nuclei. This complex was found in the nuclei after the cell membranes and cytoplasmic materials were

removed by hypotonic shock, and the rate of association of complex to cortisol was equal to the rate of nuclear association to cortisol at 37 degrees centigrade. These data about cortisol binding fit the hypothetical model for estrogen binding in target tissues.

MATERIALS AND METHODS

1. Determination of Bound Estradiol in Cytosol

For each determination, 10 female Royal Victoria Hospital strain rats, 15 days post-ovarectomy (32), 75 days of age, weighing 200-250 grams, were sacrificed by decapitation. The femorae and tibiae were cleared by blunt dissection and removed as a unit by sawing through the proximal portion of the femur (1/2 cm from the femoral head) and the distal part of the tibia (1 cm from the tibial malleolus). The distal femoral epiphyses and proximal tibial epiphyses were pried off and discarded. The outer surface of the bone was cleaned by a piece of gauze to strip off any soft tissues. The marrow elements were flushed out with ice cold saline through a needle inserted into the marrow cavity (5-10 cc/bone) (8). The saline used in this experiment is Krebs-Ringer Solution "Modified" (9 grams of sodium chloride per litre distilled water, 85.5 grams sucrose per litre, 1.42 grams of anhydrous disodium orthophosphate per litre, adjusted to pH of 7.4). The femorae and tibiae were pooled in a beaker of cold saline and all subsequent steps were performed at 0-4 degrees centigrade. Groups of six bones were cleaned of excess saline using a soft cloth and then were pulverized with an "Auto Pulverizer" (seven smashes per batch) (37). The bone chips were immediately

removed from the pulverizer and all resuspended in 30 ml saline. This was thoroughly mixed for ten minutes. The solution was filtered through glass wool into a test tube (yielding 15 cc) and this solution was centrifuged at 800 x g for ten minutes yielding the supernatant fraction and a crude nuclear pellet. The supernatant was further centrifuged at 105,000 x g for ninety minutes to obtain a mitochondrial pellet (this was discarded) and the high speed supernatant fraction (cytosol). The cytosol was analysed for calcium, hydroxyproline, hexosamine, RNA, protein, and DNA. One ml of cytosol was added to 4 test tubes in which ^{3}H-estradiol-17β (specific activity, 42 ci per mMole) and estradiol had been previously measured and dried to yield final concentration of: Tubes #1 and #2 ^{3}H-estradiol-17β 10^{-8}M (27), Tubes #3 and #4 ^{3}H-estradiol-17β 10^{-8}M and estradiol 10^{-7}M. The tubes were well mixed and let stand for 1 1/2 hours at 0-4 degrees centigrade. Separation of protein bound estradiol and free estradiol in the cytosol was accomplished by filtration in 10 ml Sephadex G-50 medium columns at 0-4 degrees centigrade. The columns were equilibrated with modified Krebs-Ringer solution. A 0.4 ml aliquot of the sample was applied on the column together with 0.05 ml each of dextran blue and chlorophenol red solutions to serve as indicator dyes. Bound estradiol was eluted in the void volume with the blue dextran, while free estradiol is retained by the gel and

is eluted with red dye (27). These columns give results corresponding to bound protein in the 8S region of sucrose density gradients (where the receptor protein is in the uterine cells for estrogen). The bound estradiol was collected in 0.5 ml aliquots which was then dissolved in a toluene scintillation solvent (Liquiflor) and counted in a Packard Tri-Carb scintillation counter. The bound estradiol in the cytosol was also estimated by charcoal absorption of free estrogen. A charcoal solution of 0.5 per cent carbon decolorizing alkaline norit A, plus 0.05 per cent dextran dissolved in tris EDTA was made. Then 0.2 ml cytosol plus 1 ml charcoal solution was mixed. The charcoal was separated by centrifugation and 0.5 ml of supernatant was eluted for counting the bound estradiol.

2. Determination of Bound Estradiol in Bone Nuclei

For each incubation, five female ovarectomized (32) rats were used. The femorae and tibiae were removed from the rats as in the previous experiment. The bones were "lightly" pulverized (1 light tap with the Auto Pulverizer) at 0-4 degrees centigrade and the large bone chips were pooled in a beaker containing 10 cc of saline plus various concentrations of ^{3}H-estradiol-17β and estradiol. This was incubated at 37 degrees centigrade for 1 1/2 hours in a Dubnoff incubator under an atmosphere of 95% oxygen

and 5% carbon dioxide. The bone was transferred to an estradiol free medium (3 washings) and all further steps were carried out at 0-4 degrees centigrade. The large bone chips were pulverized into fine pieces using seven smashes per batch (equal to six whole bones). Fifteen cc of homogenate was produced after filtration through glass wool. This was centrifuged at 800 x g for ten minutes and the crude nuclear pellet was retained. The 800 x g pellet was washed three times with 5 ml of saline, each wash was followed by centrifugation at 800 g x 10 minutes and the resulting washed pellet was called the nuclear pellet (33-35). This pellet was resuspended in saline and part was taken for DNA analysis and the other part was destined for extraction of the nuclear bound estrogen. The nuclear bound estrogen was extracted by successive washes of 5 ml of: 100 per cent ethanol, chloroform and ether (2:1), ether, and ether (19). These washes extracted all radioactivity. The washings from each sample were pooled and evaporated to dryness in scintillation vials, then redissolved in Liquiflor (10 ml) and counted. The DNA content was analysed by means of the Diphenylamine reaction for the Colorimetric Estimation of DNA (36). The results were expressed as radioactivity/mg DNA to correct for the varying amount of bone cell extraction in each experiment.

3. Determination of Bound Cortisol in Cytosol

For each incubation, 10 female Royal Victoria Hospital strain rats, 75 days of age, weighing 200-250 grams were sacrificed by decapitation. The high speed supernatant fraction (cytosol) was prepared as in experiment number one. One ml of cytosol was added to 2 test tubes containing ^{3}H-cortisol (45 ci/mMole) and 2 test tubes containing ^{3}H-cortisol and unlabelled cortisol (tenfold excess) in order to do competition studies. The tubes were well mixed and allowed to stand for 1 1/2 hours at 0-4 degrees centigrade. Separation of protein bound cortisol and free cortisol in the superantant was accomplished by filtration in 10 ml Sephadex G-50 medium columns at 0-4 degrees centigrade as in experiment #1. The bound cortisol was collected in 0.5 ml aliquots, dissolved in Liquiflor and counted in a Packard Tri-Carb scintillation counter.

4. Determination of Cortisol Uptake at Different Temperatures

The high speed supernatant fraction (cytosol) was prepared as in experiment #3. One ml of cytosol was added to 12 test tubes containing ^{3}H-cortisol (dried) to make a final concentration of ^{3}H-cortisol 10^{-8}M. Two test tubes were incubated at each of the following temperatures: 0 degrees centigrade, 25 degrees centigrade, 37 degrees centigrade, 45 degrees centigrade, 60 degrees centigrade, 100 degrees centigrade for twenty minutes

(31). Then all the test tubes were allowed to stand at 0-4 degrees centigrade for one hour and ten minutes. Columns were run on the samples and the results counted on a scintillation counter.

Blood serum was prepared by allowing rat blood to clot. This was centrifuged at 800 x g for ten minutes and the supernatant (serum) was eluted. The serum was diluted to one tenth with saline for the experiment. One ml of serum (1/10) was added to 6 test tubes containing dry ^{3}H-cortisol to make a final concentration of 10^{-8}M ^{3}H-cortisol. Two test tubes were incubated at each of the following temperatures: 0 degrees centigrade, 60 degrees centigrade, 100 degrees centigrade for twenty minutes. Then all the test tubes were allowed to stand at 0-4 degrees centigrade for seventy minutes. Columns were run on the samples and the results read on a scintillation counter.

5. Determination of Bound Cortisol in Bone Nuclei

For each incubation, five female rats were used. Large bone chips were prepared as in experiment #2. These were placed in a beaker containing 10 cc of saline plus various concentrations of ^{3}H-cortisol and unlabelled cortisol. This was incubated at 37 degrees centigrade for 1 1/2 hours in a Dubnoff incubator under an atmosphere of 95% oxygen-5% carbon dioxide. Then the bone was transferred to a cortisol free medium (3 washings).

The washed nuclear pellet was produced as per experiment #2. The resuspended pellet (in saline) was divided for DNA analysis and for extraction and measurement of nuclear bound cortisol (experiment #2). The results were expressed as radioactivity/mg DNA to correct for the varying amount of bone cell extraction in each experiment.

6. Determination of Bound Dexamethasone in Cytosol

The cytosol was prepared by the same method as experiment #3. One ml of cytosol was added to 2 test tubes containing ^{3}H-dexamethasone (25 ci/mMole) and 2 test tubes containing ^{3}H-dexamethasone and unlabelled dexamethasone (tenfold excess) in order to do competition studies. The test tubes were well mixed and let stand for 1 1/2 hours at 0-4 degrees centigrade. Columns were run on the samples, and the results read on a scintillation counter.

RESULTS

1A. Analysis of Cytosol. A high concentration of protein (1725 ug/ml) was achieved in the cytosol fraction. Absence of DNA in the cytosol signifies nuclear stability during the homogenization. The insignificant amount of RNA (22 ug/ml) suggests a preparation free of ribonucleoportein containing structures; mitochondria and polyribosomes. The small amount of calcium (3 mg%) signifies that little liberation of calcium is occurring from the raw bone surfaces. The traces of hydroxyproline (6 mg/ml) and hexosamine (20 ug/ml) are probably due to the intracellular synthesis of bone matrix (Table 1A).

1B. Bound Estradiol in the Cytosol. In each experiment, the amount of bound estradiol estimated by columns and charcoal was reliably reproduceable. Different experiments varied in the amount of bound estrogen because of differences in the amount of cytosol protein. The purpose of this experiment was to show decrease in ^{3}H-estradiol-17β uptake when unlabelled estradiol was present, signifying a specific receptor protein because of competition for the receptor sites. This difference in uptake was absent in all experiments (Table 1B).

2. Bound Estradiol in the Nucleus. No difference in ^{3}H-estradiol-17β uptake was seen when tenfold unlabelled estradiol

TABLE 1A

Analysis of Cytosol

	1	2
Calcium (mg%)	3.0	3.5
Protein (ug/ml)	1725	1675
Hydroxyproline (mg/ml)	6	4
Hexosamine (ug/ml)	20	22
RNA (ug/ml)	22	23
DNA (ug/ml)	0	0

TABLE 1B

Bound Estradiol in the Cytosol

Test tube number	1	2	3	4
Contents	^{3}H-estradiol 10^{-8}M		^{3}H-estradiol 10^{-8}M estradiol 10^{-7}M	
Experiment 1				
Columns (cpm)	224	220	253	262
Charcoal (cpm)	159	155	192	146
Experiment 11				
Columns (cpm)	442	531	515	454
Charcoal (cpm)	188	171	158	178

was in the medium (Table 2). In fact, a linear increase in uptake was seen as the concentration of ^{3}H-estradiol-17β in the medium was increased. On light microscopic analysis of the pellets, 60-70% of the volume were nuclei.

3. Bound Cortisol in the Cytosol. A 6:1 difference was seen between uptake of ^{3}H-cortisol alone and ^{3}H-cortisol mixed with a tenfold greater amount of unlabelled cortisol. This shows 83% competition (Table 3). This does not show specific uptake because the steroids with and without glucocorticoid activity were not tested for competition with the binding site.

4. Cortisol Uptake at Different Temperatures. In the cytosol, there is good uptake, up to and including 60 degrees centigrade, with a precipitous fall in uptake at 100 degrees centigrade. Similarly in the serum, there is good uptake at 0 degrees centigrade and 60 degrees centigrade, with a fall in uptake at 100 degrees centigrade (Table 4).

5. Bound Cortisol in the Nucleus. No difference in ^{3}H-cortisol uptake was seen when tenfold unlabelled cortisol was in the medium. In fact, a linear increase in uptake was seen as the concentration of ^{3}H-cortisol was increased (Table 5). On light microscopic analysis of the pellets, 60-70% of the volume were nuclei.

TABLE 2

Bound Estradiol in Nuclei

Concentration of estrogen (Moles)	Counts/mg DNA ^{3}H-estradiol-17β	Counts/mg DNA ^{3}H-estradiol-17β and estradiol
^{3}H-estradiol-10^{-9}	3080	2905
Estradiol-10^{-8}	2760	2400
	2480	2440
^{3}H-estradiol-10^{-8}	19,800	18,050
Estradiol-10^{-7}		
^{3}H-estradiol-10^{-6}	2,410,000	2,600,000
Estradiol-10^{-5}	2,110,000	2,800,000

TABLE 3

Bound Cortisol in the Cytosol

Test tube number	1	2	3	4
Contents	^{3}H-cortisol 10^{-8}M		^{3}H-cortisol 10^{-8}M cortisol 10^{-7}M	
Experiment 1				
Columns (cpm)	6,890	6,901	1,142	1,133
Experiment 2				
Columns (cpm)	7,729	7,800	1,359	1,290

TABLE 4

^{3}H-Cortisol (10^{-8}M) Uptake at Different Temperatures

Temperature Degrees C.	0	25	37	45	60	100
Cytosol						
a) Column 1 (cpm)	9,845	9,200	10,073	9,811	9,712	
Column 2 (cpm)	9,902	10,060	10,110	9,700	10,209	
b) Column 3 (cpm)	7,937				7,441	709
Column 4 (cpm)	8,038				7,584	657
Blood Serum						
Column 1 (cpm)	1,016				1,009	313
Column 2 (cpm)	974				996	361

TABLE 5

Bound Cortisol in Nuclei

Concentration of cortisol	Counts/mg DNA ^{3}H-cortisol	Counts/mg DNA ^{3}H-cortisol and cortisol
^{3}H-cortisol-10^{-8}		
Cortisol 10^{-7}	1,490	1,190
^{3}H-cortisol-10^{-7}	12,050	13,400
Cortisol-10^{-6}	12,750	12,300

6. Bound Dexamethasone in the Cytosol. There was no decrease in ^{3}H-dexamethasone uptake when unlabelled dexamethasone was present (Table 6). Thus, there is no binding site in the cytosol for which the dexamethasone competes. Another factor suggesting the absence of a cytosol binder for dexamethasone is that very little of the steroid was found in the protein bound volume of the columns (taking into account that dexamethasone has a lower specific activity).

TABLE 6

Bound Dexamethasone in the Cytosol

Test tube number	1	2	3	4
Contents	^{3}H-Dexamethasone 10^{-8}M		^{3}H-Dexamethasone 10^{-8}M dexamethasone 10^{-7}M	
Experiment 1				
Column (cpm)	135	137	138	143
Experiment 2				
Column (cpm)	130	129	125	132

DISCUSSION AND CONCLUSIONS

Bone is a difficult tissue to work with because of its large amount of calcium encasing the osteocytes. In this experiment, the method of pulverizing bone to yield osteocytes was employed (37). True nuclear pellets containing 60-70% nuclei by volume were obtained. A high concentration of cytosol protein was achieved without significant contamination by calcium, hydroxyproline, hexosamine, DNA or RNA. However, there is a problem of contamination of the cytosol fraction from blood plasma proteins. It is impossible to remove all blood from within the vessels of cortical bone by flushing the marrow cavity with saline. There are no major nutrient arteries in bone through which to perfuse the tissue such as are present in liver. This problem became apparent in the cortisol binding experiment where it was necessary to distinguish between a cytosol binding protein for the steroid or the plasma corticosteroid binding protien, transcortin. Pulverization of bone produces a low yield of cellular material. Bone is composed of a calcified organic matrix in which the cells are buried. Only those cells which are located along a line of shear are released by the pulverization procedure. Furthermore, the small amount of liberated cytoplasmic protein and nuclei (in comparison to the plentiful calcified matrix), some of the protein may

be adsorbed onto the calcified matrix. These factors are responsible for the low yield of cytoplasmic protein molecules and possible contamination of cytosol by plasma proteins.

In uterine cell cytoplasm, the plasma receptor-estrogen complex forms a protein complex sedimenting at 8S and travels with the dextran blue indicator dye in the columns (27). The binding of the hormone in target cell cytosol is at a maximum when the incubation medium concentration of estradiol is 10^{-8} molar (27). In bone cytosol, some hormone uptake was seen but this was non specific binding because of the absence of competition for binding sites (decreased radioactive hormone uptake) when unlabelled estradiol was present in the medium as well as the labelled estradiol.

In target organ estrogen uptake (20), the major portion of hormone (60-65%) was found in the nucleus and 30% appeared in the high supernatant fraction. The 5S receptor complexes were isolated in the nuclei of uterine cells. There was uptake of ^{3}H-estradiol-17β into bone nuclei but this was non specific binding. Support for the absence of specific binding is the linear increase in hormone uptake with increase of concentration of hormone in the incubation medium. This leads to the conclusion that bone is not an estrogen receptor tissue of the classical model because of the absence of estrogen specific receptor proteins in the cytosol and nucleus.

It seemed that there was a specific binding site in the cytosol for cortisol because of the positive results showing competitive uptake. The next step was to test other steroids (glucocorticoids and non-glucocorticoids) and see if these steroids caused competition for the binding site or if this site was specific for cortisol. However, at this time, it could not be ruled out that Transcortin, a plasma protein, was contaminating the cytosol and was therefore acting as the binding site. Transcortin, (29, 38, 39) or corticosteroid binding globulin, is a protein of 45,000 molecular weight and is denatured by heating at 60 degrees centigrade and has a strong affinity for cortisol.

To rule out Transcortin, an experiment was designed to denature the Transcortin at 60 degrees centigrade so that any remaining uptake would be due to a true bone cell binding site. This was successful. However, on repeating the experiment on blood serum, there was still strong cortisol uptake at 60 degrees centigrade. This is consistent with the premise that Transcortin is the contaminant in the cytosol and is the binding agent.

Finally, to rule out Transcortin, dexamethasone uptake was tested. Dexamethasone is a synthetic glucorticoid and binds specifically to hepatoma cell extract (40). Dexamethasone will not bind to rat plasma proteins (40). In this experiment, very little hormone was bound to cytosol proteins and there was no

competition. This gives conclusive evidence that the competitive uptake of cortisol was due to a plasma Transcortin contaminant in the cytosol. No cytosol receptor could be found using the dexamethasone.

Absence of specific binding was also seen in the nuclear uptake experiment. Support for non specific binding is the directly proportional increase in hormone uptake with increased concentration of hormone in the incubation medium. This experiment leads to the conclusion that bone is not a cortisol receptor tissue.

Estrogens are known to cause changes in the structure and metabolism of bones in the rat (3, 4, 7, 8). Estrogen administration causes direct effects on bone phospholipid (8). In turn, phospholipids are associated with bone formation (8, 41). Estrogen administration affects bone differently than other tissues such as liver and myocardium (8). Sakai et al. (8) suggest that it is unlikely that osseous tissue is merely taking part in a general systemic response because of a lack of accumulation of lipids in other organ systems. However, bone is a vast tissue containing precipitated salts and complex proteins which may act as a surface for non specific adsorption of lipids and hormones.

Parathyroid hormone causes loss of bone by increasing bone resorption (5, 42). Parathyroid activity is stimulated by low serum calcium (5, 42). Estrogen is known to inhibit parathyroid

hormone induced release of calcium (9) and to protect against a loss of bone mineral in immobilization osteoporosis (7). Development of immobilization osteoporosis depends on intact parathyroid glands (43). Deficiency in sex steroids will worsen osteoporosis induced by parathyroid hormone (10), possibly by increasing the sensitivity of bone to parathyroid hormone (5). The problem is whether this antagonism is a systemic or cellular phenomenon. In view of the lack of specific binding of estrogen in the bone cell, the estrogen-parathyroid antagonism would appear to be a systemic manifestation rather than a local one.

Cortisone induces osteoporosis (12, 16) due to negative calcium balance caused by gut and renal loss of calcium and due to an anti-anabolic effect on bone matrix formation. A study (17) suggested that glucocorticoids can inhibit bone resorption by a direct effect on bone because a parathyroid induced release of calcium is inhibited by low doses of dexamethasone in rat bone tissue culture. Due to the absence of specific uptake of cortisol in bone cells, the effects of cortisol on bone seem also to be a generalized systemic response.

This experiment indicates the absence of estrogen or cortisol receptor molecules in bone cells. Some factors which contribute to the absence of receptor molecules in this experimental model are: a) the concentration of receptor molecules may be very

low in bone tissues and therefore not detectable by this method; b) rough homogenization which may decrease the binding capacity of the receptor or even destroy it; and c) the three hour interval between sacrificing the animals and incubating the cytosol with hormone may decrease the binding capacity of the receptor molecule. In spite of these limiting factors, the data reported appear to be significant "negatives" in our understanding of basic bone physiology.

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