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THE HYPOCALCEMIC RESPONSE TO PROTAMINE AS A MEASURE OF BONE RESORPTION

By

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LUTHMAN, J., G. JONSON and J. PERSSON: *The hypocalcemic response to protamine as a measure of bone resorption.* Acta vet. scand. 1973, *14*, 428—435. — The heparin antagonist protamine inhibits bone resorption. In the present study protamine was given i.v. at a dose of 10 mg/kg to goats and sheep in various stages of pregnancy and lactation. It is known that bone resorption increases as pregnancy progresses. In sheep maximum in bone resportion is reached at early lactation. The hypocalcemic response to protamine followed a similar course. The slightest decrease in serum calcium was obtained during early pregnancy and the greatest during late pregnancy and early lactation. Protamine increased serum inorganic phosphorus. The increase was most pronounced during early pregnancy. Protamine did not significantly change serum magnesium.

Five lactating ewes were given 25000 i.u./kg of vitamin D_3. Serum calcium increased and the hypocalcemic response to protamine was greater after vitamin D_3 administration than before.

The results showed that the hypocalcemic response to protamine can be used as an indicator of the role of bone resorption in calcium homeostasis in various physiological situations.

protamine; bone resorption; hypocalcemia; ewes.

During recent years several findings have contributed to the hypothesis that heparin in some way is involved in bone resorption. *Goldhaber* (1965) reported that heparin potentiated the effect of parathyroid hormone on cultured bone. *Asher & Nichols* (1965) found that heparin increased bone collagenase activity. *Jaffe & Willis* (1965) and *Griffith et al.* (1965) called attention to osteoporosis and spontaneous fractures as side effects of heparin therapy in man. Heparin is synthesized in the mast cells and *Urist & McLean* (1957), *Frame & Nixon* (1968) and *Rockoff & Armstrong* (1970) found increased number of mast cells in endosteum and bone marrow during conditions where bone resorption was increased.

Anderson et al. (1967) obtained hypocalcemic effect in man of the heparin antagonist protamine. The authors suggested that protamine caused hypocalcemia by inhibiting the bone resorption enhancement property of heparin. Later *Johnston et al.* (1970) showed in controlled experiments in rats that protamine inhibits bone resorption.

The hypocalcemic effect of protamine was recently studied in sheep (*Luthman et al.* 1973). As could be expected of a drug inhibiting bone resorption the hypocalcemic effect was greater in lambs than in adult animals.

Drugs with calcitonin-like activity may be used to study the role of bone resorption in calcium homeostasis in various physiological conditions, as the effect of such drugs reflects the rate of bone resorption. Protamine seems most suitable in this respect. It is cheap, easily available and gives no side reactions.

The aim of the present investigation was to study the hypocalcemic effect of protamine in goats and sheep. The calcium requirement increases during pregnancy, and despite increased intestinal resorption, bone resorption is largely increased during late pregnancy (*Braithwaite et al.* 1970). Pregnant and lactating animals therefore seemed to be convenient subjects in testing the usefulness of the hypocalcemic effect of protamine as an indicator of bone resorption.

MATERIAL AND METHODS

Five ewes of the Swedish landrace breed were used. The animals were kept indoors and fed hay and mineralized and vitaminized concentrate. Calcium uptake during late pregnancy was calculated to be about 9 g/day. The animals were given repeated i.v. injections of protamine chloride (Protamin, 10 mg/ml, Vitrum, Stockholm, Sweden). The dose used was 10 mg/kg. Blood was sampled 1, 2 and 4 hrs. after the injection. The first protamine injection was given about three weeks after conception, and the second two weeks before lambing. The third and fourth injections were given 12 and 42—54 days after lambing. One day after the last study an i.m. injection of vitamin D_3 was given in order to further increase bone resorption. The dose used was 25000 i.u./kg. Protamine was then administered after three days.

The goats were kept under the same conditions as the sheep. The calculated calcium uptake during late pregnancy was 6 g/day. Four goats were originally available, but at the time of the second protamine injection only three goats could be used. Two other goats were therefore included in the study. Protamine was given during the first and fifth months of pregnancy and 12 days post partum. The goats were not milked regularly.

Serum calcium was determined according to *Skerry* (1965). Serum inorganic phosphorus was analyzed according to *Fiske & Subbarow* (1925). Serum magnesium was determined by means of commercial reagents (Merckotest Magnesium, E. Merck, Darmstadt, Germany).

RESULTS AND DISCUSSION

The greatest decrease in serum calcium was in most cases obtained 2 hrs. after protamine administration. As seen from Table 1 the decrease was very slight during the first month of

Table 1. Changes in the serum levels of calcium, inorganic phosphorus and magnesium in sheep after protamine injection (10 mg/kg). n = 5.

	Pretreatment level	Hours		
		1	2	4
			△ Ca (mg/100 ml)	
1st month of pregnancy	9.2±0.2	—0.1±0.2	—0.3±0.1	—0.4±0.2
5th „ „ „	9.9±0.3	—1.2±0.6*	—1.6±0.5**	—1.8±0.7*
12 days post partum	9.9±0.5	—1.4±0.7**	—1.8±0.7**	—0.9±0.6*
42—54 days post partum	9.5±0.4	—0.6±0.2*	—1.1±0.5*	—0.6±1.0
			△ P (mg/100 ml)	
1st month of pregnancy	4.7±1.4	+2.8±0.9	+1.8±1.2	+1.86±0.9
5th „ „ „	4.7±0.6	+1.2±0.8*	+0.7±0.4	—0.58±0.3**
12 days post partum	4.5±1.0	+0.8±1.2**	+1.0±1.0	+0.3±1.4
42—54 days post partum	4.6±0.7	+0.1±0.6*	+0.3±0.9	—0.2±1.1**
			△ Mg (mg/100 ml)	
1st month of pregnancy	2.8±0.3	—0.2±0.1	—0.2±0.1	—0.2±0.1
5th „ „ „	2.9±0.4	—0.1±0.1	—0.2±0.1	—0.2±0.2
12 days post partum	2.6±0.3	—0.2±0.1	—0.3±0.1	—0.3±0.1
42—54 days post partum	2.5±0.3	0.0±0.1	—0.1±0.2	—0.1±0.1

* almost significantly different from corresponding value during 1st month of pregnancy ($0.05 > P > 0.01$).

** significantly different from corresponding value during 1st month of pregnancy ($0.01 > P > 0.001$).

pregnancy. The calcium response to protamine then gradually increased and was greatest in late pregnancy and early lactation. The decreases in serum calcium obtained during the fifth month of pregnancy and 12 days post partum differed significantly ($0.01 > P > 0.001$) from that obtained during the first month of pregnancy. One to two months post partum the response was lesser, but almost significantly greater than during early pregnancy ($0.05 > P > 0.01$).

Comprehensive studies on calcium metabolism in pregnant and lactating ewes have been published by *Braithwaite et al.* (1969, 1970, 1972). The transfer of calcium to the fetuses increases markedly from about the second month of pregnancy, and maximum is reached at parturition. There is no exchange between the fetal and maternal pools of calcium. The transfer of calcium across the placenta is thus a one-way process. During pregnancy the intestinal absorption of calcium is increased, but increased bone resorption is necessary to meet the requirement. During late pregnancy the amount of calcium mobilized from the skeleton is about equal to that absorbed from the intestine. The ewe is in negative calcium balance from about the fourth month of pregnancy. It is during the last month of pregnancy hypocalcemic paresis appears (*Jonson et al.* 1971, 1973). *Braithwaite et al.* (1969) found bone resorption to be greatest at the height of milk production, which usually occurs two to three weeks post partum. Bone resorption decreases as milk production decreases, and the ewe comes into positive calcium balance about 30 days post partum.

Serum inorganic phosphorus increased after protamine injection. The increase was greatest during early pregnancy and least one to two months post partum. Similar results were obtained earlier (*Luthman et al.* 1973), and it was suggested that protamine must affect phosphate metabolism also in other ways than by inhibiting bone resorption. It is possible that a redistribution of phosphate occurs and that phosphate ions move into the vascular space.

Serum magnesium decreased slightly, but there were no significant differences in response between the various stages of pregnancy and lactation.

The effects of protamine in the goats are shown in Table 2. Also in the goats the decrease in serum calcium was greatest 12 days post partum. The decreases were somewhat lesser in the

Table 2. Changes in the serum levels of calcium, inorganic phosphorus and magnesium in goats after protamine injection (10 mg/kg).

	Pretreatment level	Hours		
		1	2	4
		△ Ca (mg/100 ml)		
1st month of pregnancy (n=4)	9.5±0.2	—0.3±0.0	—0.5±0.2	—0.8±0.3
5th „ „ „ (n=5)	9.8±0.3	—0.7±0.5	—1.1±0.2	—0.8±0.4
12 days post partum (n=5)	9.2±0.3	—1.0±0.2	—1.3±0.4	—1.0±0.5
		△ P (mg/100 ml)		
1st month of pregnancy	5.7±1.1	+1.2±0.6	+0.5±0.4	+0.8±0.9
5th „ „ „	6.5±0.9	—0.2±1.2	—0.2±2.1	—1.3±2.1
12 days post partum	5.9±1.0	+1.0±1.6	+3.2±2.2	+1.5±1.5
		△ Mg (mg/100 ml)		
1st month of pregnancy	2.3±0.0	—0.1±0.1	+0.1±0.1	0.0±0.1
5th „ „ „	2.5±0.3	—0.1±0.1	—0.2±0.1	—0.2±0.2
12 days post partum	2.4±0.4	—0.2±0.1	—0.3±0.1	—0.3±0.2

goats than in the sheep. All goats had single fetuses, while all the ewes bore twins.

It is well known that pharmacological doses of vitamin D_3 increase bone resorption. As seen from Table 3 serum calcium was higher ($0.05 > P > 0.01$) three days after vitamin D_3 treatment. The levels of inorganic phosphorus and magnesium did not change significantly.

The serum calcium response to protamine after vitamin D_3 treatment is shown in Fig. 1. Protamine caused a greater fall in

Table 3. Changes in serum calcium, inorganic phosphorus and magnesium in lactating sheep after i.m. administration of 25000 i.u./kg of vitamin D_3.

	mg/100 ml		
	Ca	P	Mg
Before vitamin D_3 administration	9.5±0.4	4.6±0.7	2.5±0.3
Three days after vitamin D_3 administration	10.3±0.5*	5.3±1.5	2.4±0.3

* almost significantly different from pretreatment level ($0.05 > P > 0.01$).

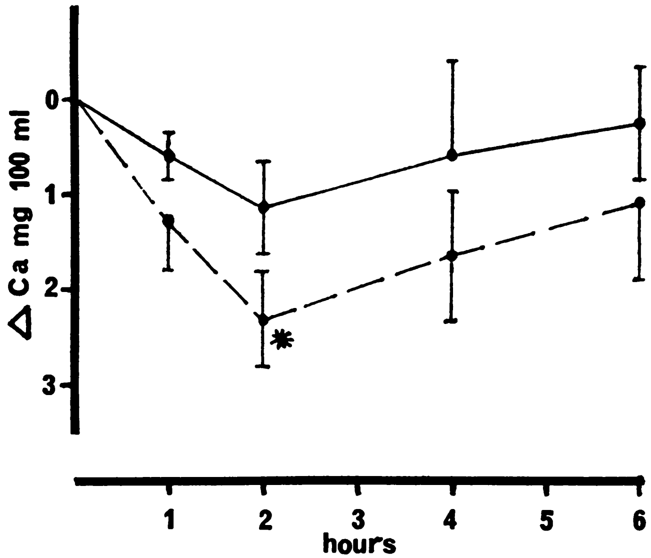

Figure 1. The hypocalcemic response to protamine (10 mg/kg i.v.) in lactating ewes before ——— and three days after administration of 25000 i.u./kg of vitamin D_3 -------.

serum calcium three days after vitamin D_3 treatment than before ($0.05 > P > 0.01$). The curve in Fig. 1 illustrating the calcium response before vitamin D_3 administration is a graphic representation of the values given in Table 1, 42 to 54 days post partum. The inorganic phosphorus and magnesium responses did not change significantly after vitamin D_3 administration.

The results obtained in the present investigation showed that protamine can be used in the study of the role of bone resorption in calcium homeostasis. The hypocalcemia caused by protamine was most pronounced in the situations where bone resorption is known to be most accelerated.

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SAMMANFATTNING

Det hypokalcemiska svaret på protamininjektion som ett mått på benresorbtionen.

Heparinantagonisten protamin blockerar benresorbtionen. Protamin gavs intravenöst (10 mg/kg) till får och getter i olika dräktighets- och laktationsstadier. Det är känt att benresorbtionen ökar allt eftersom dräktigheten framskrider. Hos får når benresorbtionen maximum under tidig laktation. Den minsta sänkningen av serumkalcium efter protamininjektion erhölls under tidig dräktighet och den största under sen dräktighet och tidig laktation.

Protamin ökade serumkoncentrationen av oorganisk fosfor. Ökningen var mest påtaglig under tidig dräktighet.

Serumkoncentrationen av magnesium förblev i stort sett oförändrad efter protamininjektion.

Fem lakterande tackor behandlades med 25000 I.E. vitamin D_3/kg. Serumkoncentrationen av kalcium ökade, och det hypocalcemiska svaret på protamininjektion var större efter D-vitamin behandlingen.

Resultaten visade att protamin kan användas för att studera den roll benresorbtionen spelar i kalciumhomeostasen under olika fysiologiska förhållanden.

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