島根大農研報 (Bull. Fac. Agr. Shimane Univ.) 23 : 204−208, 1989

Effects of Calcitonin Administration on Urinary Calcium and Phosphorus Excretion in Sheep

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カルシトニン投与がめん羊の尿中カルシウムおよびリン排泄に及ぼす影響

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Summary

The objective of this experiment is to investigate roles of calcitonin on urinary calcium and phosphorus excretion in sheep. Three thyroidectomized wethers were injected with 0 (vehicle), 0.25, 0.5 and 1 U/kg body weight of porcine calcitonin and three sham operated sheep were injected with vehicle. Serum calcium and phosphorus concentrations were decreased by calcitonin administration. And serum parathyroid hormone concentration was increased along with the reduction of serum calcium concentration. Urinary volume was more in thyroidectomized sheep than in sham operated ones and tended to be decreased by calcitonin administration though the reduction of urinary volume was not dose dependent. Urinary calcium excretion was more in thyroidectomized sheep than in sham operated ones. Though the smallest does of calcitonin decreased calcium excretion in urine, the largest dose increased urinary calcium excretion in thyroidectomized sheep. Urinary phosphorus excretion was much less in thyroidectomized animals than in sham operated ones. Calcitonin administration increased phosphorus excretion in dose dependent manner in thyroidectomized sheep. However any doses of calcitonin could not recover phosphorus excretion to the level of sham operated sheep. These results suggested that calcitonin commonly decreased urinary calcium excretion for the conservation of calcium, while a high concentration of blood calcitonin increased urinary calcium excretion which promotes the hypocalcemic action of calcitonin. And it was confirmed that calcitonin had a hyperphosphaturic action in sheep.

Introduction

The hypocalcemic hormone, calcitonin, affects urinary mineral excretion. RASMUSSEN *et al.*[1] indicated that calcitonin administration decreased urinary calcium excretion in rats. On the other hand, BARLET[2] reported that calcitonin infusion increased urinary calcium and phosphorus excretion in sheep. And several investigators obtained different

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results about the effect of calcitonin administration on urinary calcium excretion.[3,4]

In the previous study, we showed that calcitonin deficiency increased calcium excretion in urine and decreased phosphorus excretion in sheep.[5] Furthermore, it was shown that urinary calcium excretion was increased more in thyroidectomized sheep than in intact ones by a oral calcium load.[6]

The present study was designed to investigate effects of several doses of calcitonin on urinary calcium and phosphorus excretion in thyroidectomized sheep.

Materials and Methods

Six young wethers weighing about 25 kg were used. All animals were kept in metabolism crates. Three sheep were thyroidectomized and the other three were performed sham operation two weeks before the experiment. Thyroidectomized animals were intramuscularly injected with 0.25 mg of L-thyroxine (Nakarai Chemicals Ltd.,Kyoto) dissolved in corn oil daily after the operation was done. All sheep were given a diet shown in Table 1 at a level of 2% of body weight daily. Water was available at all times.

Table-1 Composition of diet

Ingredient	
Orchardgrass hay	59.7
Ground barley	30.0
Soybean meal	9.8
Sodium chloride	0.3
Calcium carbonate	0.2

% of air dry matter

Thyroidectomized sheep were intramuscularly injected with 0, 0.25, 0.5 and 1 U/kg body weight of porcine calcitonin (Armor Pharmaceutical Co., Eastbourne, England; 80 U/mg protein) dissolved in 16% gelatin at 6 day intervals at feeding time. At the same time, sham operated sheep were injected with the vehicle.

Blood samples were collected 8 hours after injection, and urine collection were made over 24 hours after injection. Serum and urinary calcium concentrations were measured by an atomic absorption spectrophotometry and phosphorus concentrations were measured by the method of Gomori.[7] Serum parathyroid hormone concentrations were determined by a radioimmunoassay using bovine parathyroid hormone.[8] Statistical differences were evaluated by Student't-test.

Results and Discussion

As shown in Table 2, serum calcium concentration tended to be decreased by calcitonin administrations in thyroidectomized sheep and the reduction became significant ($p<0.05$) when animals were injected with 0.5 U/kg body weight of calcitonin. Serum phosphorus concentration was also decreased by calcitonin administrations while there was no significant difference between calcitonin and vehicle injection in thyroidectomized animals. It seemed that a large variation of serum phosphorus concentration masked a substantial difference between treatments.

Serum parathyroid hormone concentrations tended to be higher in sham operated

Table-2 Effect of calcitonin injection on serum calcium, phosphorus and parathyroid hormone concentration in thyroidectomized sheep

	Sham operated sheep	Amount of calcitonin injection into thyroidectomized sheep (U/kg)			
		0	0.25	0.50	1.00
Calcium (mg/100 ml)	9.52±0.31	9.68±0.11	8.50±1.36[a]	8.47±0.69[b]*	7.40±0.39[b]**
Phosphorus (mg/100 ml)	6.64±0.32	6.60±1.04	5.73±0.46[b]	5.52±1.16[a]	5.29±1.05[b]
Parathyroid hormone (U/l)	3.03±0.56	2.48±0.30	3.00±0.71	3.41±0.68	3.69±0.62*

Values were indicated means±SD for 3 thyroidecomized sheep and for 3 times replication of 3 sham operated sheep.
Sham operated sheep were injected with vehicle.
* ; $p<0.05$, ** ; $p<0.01$ compared with the value when thyroidectomized sheep were injected 0 U/kg of calcitonin (vehicle).
a ; $p<0.05$, b ; $p<0.01$ compared with the value of sham operated sheep.

Table-3. Effect of calcitonin injection on urinary volume, calcium and phosphorus excretion in thyroidectomized sheep

	Sham operated sheep	Amount of calcitonin injection into thyroidectomized sheep (U/kg)			
		0	0.25	0.50	1.00
Urinary volume (l/day)	1.06±0.59	1.81±0.61	0.92±0.45	1.37±0.35	1.24±0.68
Calcium (mg/day)	18.7±8.7	66.3±13.6[b]	27.2±11.9*	39.1±17.0[a]	98.6±30.5[b]
Phosphorus (mg/day)	46.0±6.5	10.2±3.9[b]	14.3±3.8[b]	16.9±3.6[b]	19.1±4.2*[b]

Values were indicated means±SD for 3 thyroidecomized sheep and for 3 times replication of 3 sham operat ed sheep.
Sham operated sheep were injected with vehicle.
* ; $p<0.05$ compared with the value when thyroidectomized sheep were injected 0 U/kg of calcitonin (vehicle).
a ; $p<0.05$, b ; $p<0.01$ compared with the value of sham operated sheep.

sheep than in thyroidectomized ones which were injected with vehicle. These results were consistent with a previous report.[6] By calcitonin administrations, serum parathyroid hormone levels were increased along with the reduction of serum calcium concentrations. Hypocalcemia is well known to stimulate parathyroid hormone secretion.[9] It is natural to consider that the hypocalcemia, induced by calcitonin administrations, increases serum parathyroid hormone concentration in this experiment.

Urinary volume was not different between sham operated and thyroidectomized sheep which were injected vehicle (Table 3). Calcitonin injection tended to decrease urinary volume although the reduction of urinary volume was not dose dependent. Barlet[2] reported that calcitonin infusion (20 mU/kg body weight/hour) increased urinary volume in sheep. On the other hand, Kimura and Ogata[10] indicated that a large dose of calcitonin (1-1.3 U/rat) increased urinary volume but a small dose of calcitonin injection (2 mU/rat) did not change urinary volume in spite of affecting urinary mineral excretion. The results from the present study suggests that calcitonin adminisration may decrease urinary volume in sheep.

Urinary calcium excretion was significantly ($p<0.05$) more in thyroidectomized sheep than in sham operated ones when they were injected with vehicle. In the previous

experiment,[5] urinary calcium excretion was found to be larger in thyroidectomized sheep compared with intact sheep. Calcium excretion was decreased by the smallest dose of calcitonin in thyroidectomized animals and the significant difference of calcium excretion between sham operated and thyroidectomized sheep disappeared. TALMAGE *et al*[11] showed that the reduced calcium storage in animal body by thyroidectomy increased urinary calcium and that calcitonin injection stimulated the skeletal retention of calcium, which decreased urinary calcium excretion. They suggested that one of the roles of calcitonin was storage of calcium in the skeleton. The reduction of urinary calcium excretion may reflect the increment in restoring calcium in the bone of sheep injected with 0.25 U/kg body weight of calcitonin. The consideration may be supported by the results that the same dose of calcitonin administration decreases urinary hydroxyproline excretion which was thought to be an indicator of bone resorption.[12]

Urinary calcium excretion, however, increased as larger doses of calcitonin were injected in thyroidectomized sheep. It is clear that calcitonin has a hypercalcuric action in sheep. The largest dose of calcitonin increased serum parathyroid hormone which was known to decrease calcium excretion.[13] From these results, it is considerable that the hypercalcuric action of calcitonin overcomes the suppressive effect of parathyroid hormone on calcium excretion. KIMURA and OGATA[10] found that a small dose of calcitonin administration decreased calcium excretion in urine but a larger dose of calcitonin increased calcium excretion in rats. The results were consistent with the present results using sheep. These facts suggest that calcitonin plays two kinds of role related to urinary calcium excretion, i. e., 1) calcitonin usually stimulates storage of calcium which reduces urinary calcium excretion and 2) calcitonin increases urinary calcium excretion when blood calcitonin level is high, which promotes it's hypocalcemic action. However, it was questionable whether the latter action was physiological or not because thyroidectomy increased urinary calcium excretion and oral calcium load found to increase more urinary calcium excretion in thyroidectomized sheep than in sham operated ones.[6]

Phosphorus excretion in urine was much less ($p<0.01$) in thyroidectomized sheep than in sham operated ones when they were injected with vehicle. Calcitonin administration tended to increase urinary phosphorus excretion in dose dependent manner. Some investigators reported the hyperphosphaturic effect of calcitonin in various animals.[1,2,10] However, urinary phosphorus excretion was still more in sham operated sheep than in thyroidectomized sheep injected with the largest dose of calcitonin. It is not clear why calcitonin injection can not completely recover urinary phosphorus excretion in thyroidectomized sheep. There may be a possibility that chronic calcitonin deficiency reduces the reactivity of urinary phosphorus excretion to calcitonin.

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摘　　要

カルシトニンがめん羊の尿中カルシウムおよびリン排泄に及ぼす影響を検討するために，甲状腺摘除を行なった去勢雄羊に対し，体重1kgあたり0，0.25，0.50，1.00国際単位のブタカルシトニンを筋注投与した．また疑似手術めん羊には，溶剤のみを投与した．カルシトニンの投与量が増加するにつれて，血清中カルシウム濃度は有意に低下し，リン濃度は低下傾向を示した．また血清中上皮小体ホルモン濃度はカルシウム濃度の低下に伴い上昇した．

尿量はカルシトニン投与により低下傾向を示したが，投与量との間に関係は認められなかった．尿中カルシウム排泄量は，疑似手術めん羊と比較し，甲状腺摘除めん羊で多かった．0.25単位のカルシトニン投与により，尿中カルシウム排泄量は低下したが，1単位のカルシトニン投与では逆に増加した．尿中リン排泄量似手術めん羊と比較し，甲状腺摘除めん羊で著しく少なかった．甲状腺摘除めん羊ではカルシトニンの投与は疑量が増すにつれ，リン排泄量は増加傾向を示したが，1単位のカルシトニン投与においても，疑似手術めん羊と比較しリン排泄量は非常に少なかった．

以上の結果からカルシトニンは通常尿中カルシウム排泄を低下することにより，カルシウム蓄積を促進するが，血中カルシトニン濃度が著しく増加すると逆にカルシウム排泄を促進することが示唆された．またカルシトニンは尿中リン排泄を増加させることが明かとなった．