Salmon Calcitonin-Induced Stimulation of $1\alpha,25$ -Dihydroxycholecalciferol Synthesis in Rats Involving a Mechanism Independent of Adenosine 3':5'-Cyclic Monophosphate

By Noboru HORIUCHI,* Hiroo TAKAHASHI†, Toshio MATSUMOTO,‡ Naoyuki TAKAHASHI,* Eiichiro SHIMAZAWA†, Tatsuo SUDA* and Etsuro OGATA§

*Department of Biochemistry, Showa University Dental School, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142,

†Department of Pharmacology, Teikoku Hormone Manufacturing Co., Kawasaki, Kanagawa 213, ‡First Department of Medicine, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, and §Department of Medicine, University of Tsukuba, Niihari, Ibaraki 300-31, Japan

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The effect of natural salmon calcitonin on accumulation in plasma of $1\alpha,25$ -dihydroxy- $[^3\text{H}]$ cholecalciferol from 25-hydroxy $[^3\text{H}]$ cholecalciferol *in vivo* was investigated in vitamin D-deficient thyroparathyroidectomized rats into which graded doses of the hormone were continuously infused by use of a balance study system. A dose-dependent increase in plasma concentrations of $1\alpha,25$ -dihydroxy $[^3\text{H}]$ cholecalciferol was observed with calcitonin infusion for 6–30 h at a rate greater than 20 M.R.C. m-units/h. Infusion of parathyrin or cyclic AMP produced a similar stimulation [Horiuchi, Suda, Takahashi, Shimazawa & Ogata (1977) *Endocrinology* 101, 969–974], but the maximal effect of calcitonin was additive to that of either parathyrin or cyclic AMP. Furthermore concurrent infusion of theophylline (0.5 $\mu\text{mol/h}$) did not potentiate the effect of submaximal doses (3 and 20 M.R.C. m-units/h) of calcitonin. Plasma concentrations of calcium showed a decrease with calcitonin infusion for 30 h, but those of P_i remained unchanged. These results strongly suggest that the rat kidney is endowed with a calcitonin-sensitive 1α -hydroxylase system that is separate from the parathyrin/cyclic AMP system and is independent of changes in plasma P_i .

In mammals, plasma calcium homeostasis is efficiently maintained by three calcium-regulating hormones [parathyrin, calcitonin and $1\alpha,25(\text{OH})_2\text{D}_3$]. It is well known that the conversion of $25(\text{OH})\text{D}_3$ into $1\alpha,25(\text{OH})_2\text{D}_3$ takes place exclusively in the kidney (Fraser & Kodicek, 1970). The renal biosynthesis of $1\alpha,25(\text{OH})_2\text{D}_3$ is under strict control by the serum calcium concentration through parathyrin (Boyle *et al.*, 1971; Garabedian *et al.*, 1972; Fraser & Kodicek, 1973; Hughes *et al.*, 1975), by the serum P_i concentration (Tanaka & DeLuca, 1973; Hughes *et al.*, 1975), by vitamin D status in the organism (Tanaka & DeLuca, 1974; Horiuchi *et al.*, 1974), and by other modulators (Tanaka *et al.*, 1976; Spanos *et al.*, 1976, 1978b). Parathyrin and the stress of hypophosphataemia independently promote the production of $1\alpha,25(\text{OH})_2\text{D}_3$ (Tanaka & DeLuca, 1973; Hughes *et al.*, 1975). $1\alpha,25(\text{OH})_2\text{D}_3$ inhibits renal $25(\text{OH})\text{D}_3$ 1α -hydroxylase activity directly (Spanos *et al.*, 1978a) or indirectly by suppressing

endogenous secretion of parathyrin (Chertow *et al.*, 1975; Horiuchi *et al.*, 1976). These findings suggest at least in part the existence of a negative-feedback mechanism, in which parathyrin stimulates the synthesis of $1\alpha,25(\text{OH})_2\text{D}_3$, which in turn suppresses parathyrin secretion (Brumbaugh *et al.*, 1975; Suda *et al.*, 1977).

Another calcium-regulating hormone, calcitonin, was shown to enhance renal conversion of $25(\text{OH})\text{D}_3$ into $1\alpha,25(\text{OH})_2\text{D}_3$ in vitamin D-deficient rats (Galante *et al.*, 1972; Lorenc *et al.*, 1977). The latter group, however, showed that the hormone did not exhibit any effect when the animals were thyroparathyroidectomized. They concluded that the stimulatory effect of calcitonin was mediated by endogenous secretion of parathyrin. The effect of calcitonin on $1\alpha,25(\text{OH})_2\text{D}_3$ synthesis and the interaction with that of parathyrin appear of considerable interest, in view of the facts that the stimulatory effect of parathyrin on $1\alpha,25(\text{OH})_2\text{D}_3$ synthesis appears to be mediated by cyclic AMP (Horiuchi *et al.*, 1977) and that calcitonin, although counteracting parathyrin in plasma calcium homeostasis, induces, like parathyrin, a stimulation of the renal

Abbreviations used: $25(\text{OH})\text{D}_3$, 25-hydroxycholecalciferol; $1\alpha,25(\text{OH})_2\text{D}_3$, $1\alpha,25$ -dihydroxycholecalciferol; $24,25(\text{OH})_2\text{D}_3$, $24,25$ -dihydroxycholecalciferol.

cyclic AMP system even in vitamin D-deficient rats (Kakuta *et al.*, 1978).

In the present study, we re-examined the effect of calcitonin on the conversion of $25(\text{OH})\text{D}_3$ into $1\alpha,25(\text{OH})_2\text{D}_3$ in vitamin D-deficient thyroparathyroidectomized rats, into which calcium and calcitonin were constantly infused. Calcitonin enhanced the conversion of $25(\text{OH})\text{D}_3$ into $1\alpha,25(\text{OH})_2\text{D}_3$ even in vitamin D-deficient thyroparathyroidectomized animals. This stimulatory effect of calcitonin was additive to that of parathyrin or cyclic AMP, suggesting the existence of a calcitonin-sensitive 1α -hydroxylase system that is independent of the parathyrin-dependent one.

Materials and Methods

Animals

Male weanling rats (Sprague-Dawley) weighing 50 g were maintained on a synthetic vitamin D-deficient diet containing 0.45% calcium and 0.3% phosphorus (Suda *et al.*, 1970). They were supplemented three times weekly with vitamins A, E and K in cotton seed oil. At 6 weeks, when they were severely hypocalcaemic (5–6 mg/100 ml of plasma) and growth was retarded [body wt. 180 ± 20 g (mean $\pm$ S.E.) compared with 270 ± 30 g (mean $\pm$ S.E.; $n = 20$) in vitamin D-repleted animals], the following experiments were performed.

Protocol of infusion experiments

The animals were thyroparathyroidectomized under anaesthesia with hexobarbital (100 mg/kg body wt.) injected intraperitoneally. The left femoral vein was cannulated with a heparin-treated polyethylene tube (Intramedic 7400). Each animal was loosely fixed in a Ballman cage in a plastic chamber controlled at 24°C with about 50% humidity. All of these surgical procedures were completed in about 30 min. The rats were immediately infused at a rate of 3 ml/h via the cannulated femoral vein with a solution composed of 5 or 10 mM- CaCl_2 , 5 mM- MgCl_2 , 20 mM- NaCl , 2.5 mM- KCl and 4% glucose (Koide *et al.*, 1976). In addition, graded amounts of bovine parathyrin (trichloroacetic acid-treated powder, 218 U.S.P. units/mg; Wilson Laboratories, Chicago, IL, U.S.A.), natural salmon calcitonin (1300 M.R.C. units/mg; a gift from Dr. M. Sawai, Teikoku Hormone Manufacturing Co., Kawasaki, Japan), synthetic salmon calcitonin (400 M.R.C. units/mg; a gift from the Medical Research Council, London, U.K.), cyclic AMP (Sigma) and theophylline (Nakarai Chemical Co., Kyoto, Japan) were constantly infused, individually or in combinations, into the thyroparathyroidectomized rats immediately or starting at indicated times after the surgery.

Analysis of $25(\text{OH})\text{D}_3$ metabolism

At 24 h after the surgery, 45 pmol (0.5 μCi) of $25(\text{OH})[26(27)\text{-}^3\text{H}]\text{D}_3$ (The Radiochemical Centre, Amersham, Bucks., U.K.) in 0.05 ml of ethanol was injected via the cannulated femoral vein. The rats were killed 6 h later and their blood was collected. The blood plasma was extracted with methanol and chloroform (Bligh & Dyer, 1959). Extracts were applied to a column (1.5 cm $\times$ 30 cm) of Sephadex LH-20. The columns were equilibrated and eluted with a mixed solvent of 65% chloroform and 35% *n*-hexane (Holick & DeLuca, 1971). Results are expressed as the circulating concentrations of $1\alpha,25(\text{OH})_2[^3\text{H}]\text{D}_3$ derived from $25(\text{OH})[^3\text{H}]\text{D}_3$.

Identification of $1\alpha,25(\text{OH})_2\text{D}_3$

The radioactive $1\alpha,25(\text{OH})_2\text{D}_3$ obtained from Sephadex LH-20 columns was identified by co-chromatography with authentic $1\alpha,25(\text{OH})_2\text{D}_3$ (a gift from Dr. I. Matsunaga, Chugai Pharmaceutical Co., Tokyo, Japan) on a Waters model 204 high-pressure liquid chromatograph, equipped with a stainless-steel column (15 cm $\times$ 4.6 mm) packed with Zorbax-SIL. The column was eluted with a solvent of 3% methanol in dichloromethane at a flow rate of 1.0 ml/min (Ikekawa & Koizumi, 1976). The radioactive $1\alpha,25(\text{OH})_2\text{D}_3$ from Sephadex LH-20 columns was also identified by periodate cleavage. The $1\alpha,25(\text{OH})_2[^3\text{H}]\text{D}_3$ fraction was evaporated to dryness, dissolved in 2 ml of methanol and treated with 1 ml of 5% NaIO_4 . The reaction was carried out at room temperature overnight. The reaction mixture was extracted with chloroform and the radioactivities of the extracts were determined.

Measurements of plasma calcium and P_i concentrations were by the procedures previously described (Horiuchi *et al.*, 1974). Statistical significance was determined by analysis of variance or Student's *t* test (Sakuma, 1964).

Results

Fig. 1(a) shows a Sephadex LH-20 column-chromatographic profile of chloroform extracts of blood plasma from vitamin D-deficient thyroparathyroidectomized rats infused with 500 M.R.C. m-units of calcitonin/h for 30 h immediately after thyroparathyroidectomy. The rats were injected intravenously with 0.5 μCi of $25(\text{OH})[^3\text{H}]\text{D}_3$ 24 h after thyroparathyroidectomy and were killed 6 h thereafter. $25(\text{OH})[^3\text{H}]\text{D}_3$ was converted into polar metabolites. Over 90% of the radioactivity of the polar peak from the Sephadex LH-20 column migrated to exactly the same position as authentic $1\alpha,25(\text{OH})_2\text{D}_3$ on a high-pressure liquid chromatograph (Fig. 1b). Furthermore, 88% of the radioactivity of the $1\alpha,25(\text{OH})_2\text{D}_3$ fraction from the Sephadex LH-20 column was recovered in chloro-

Fig. 1. Chromatographic analysis of extracts of blood plasma from vitamin D-deficient thyroparathyroidectomized rats infused with 500 m-units of calcitonin/h for 30 h

Calcitonin was infused immediately after thyroparathyroidectomy. (a) Sephadex LH-20 column profile of plasma 6 h after $25(\text{OH})[^3\text{H}]\text{D}_3$ injection. (b) High-pressure liquid chromatograph of the $1\alpha,25(\text{OH})_2\text{D}_3$ fraction collected from the Sephadex LH-20 column shown in (a). Before application to the column, 300 ng of authentic $1\alpha,25(\text{OH})_2\text{D}_3$ was added to the sample. In (b) the solid line shows A_{254} and the bars represent radioactivity.

form extracts after periodate cleavage. It was therefore assumed that the radioactive polar peak on the Sephadex LH-20 column was mainly $1\alpha,25(\text{OH})_2\text{D}_3$.

Fig. 2. Time course of change in the accumulation in vivo of $1\alpha,25(\text{OH})_2[^3\text{H}]\text{D}_3$ (a) and calcium concentrations (b) in plasma by calcitonin infused at a rate of 500 m-units/h

The experimental group of rats (○) were first infused with the medium containing 5 mM- CaCl_2 for 24, 10 or 0 h, and then with calcitonin dissolved in the same medium containing 10 mM- CaCl_2 for 6, 20 or 30 h respectively. Respective control rats (●) at each point were also infused with 5 mM- CaCl_2 for the first 24, 10 or 0 h, and switched to the same medium containing 10 mM- CaCl_2 for 6, 20 or 30 h respectively. The respective control results were plotted at the time points of 6, 20 or 30 h of calcitonin infusion. The animals whose results are indicated at the zero time were infused with 5 mM- CaCl_2 for 30 h after thyroparathyroidectomy. All rats were injected intravenously with $0.5 \mu\text{Ci}$ (45 pmol) of $25(\text{OH})[^3\text{H}]\text{D}_3$ 24 h after thyroparathyroidectomy, and blood was collected 6 h thereafter. The vertical bar represents the S.E.M., and the numbers in parentheses indicate the number of animals. Results that are significantly different from their respective control values are indicated as follows: * $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$.

Infusion of calcitonin into vitamin D-deficient thyroparathyroidectomized rats did not allow them to survive, unless calcium was concomitantly supplied. With concurrent infusions of 10mM-CaCl₂ the animals were able to survive for a considerable period of time. This system was also useful for maintaining constant plasma concentrations of various hormones with short half lives. Fig. 2 shows the time course of change in the accumulation of 1 α ,25-(OH)₂[³H]D₃ and calcium concentrations in blood plasma after initiation of the calcitonin infusion at a rate of 500m-units/h into the vitamin D-deficient thyroparathyroidectomized rats. The animals were given 25(OH)[³H]D₃ 6h before the indicated times. The plasma concentrations of 1 α ,25-(OH)₂[³H]D₃ were enhanced significantly ($P < 0.01$) 6–30h after the initiation of continuous infusion of calcitonin, compared with that of vitamin D-deficient thyroparathyroidectomized rats infused with 10mM-CaCl₂ for 30h (Fig. 2a). Plasma concentrations of calcium (Fig. 2b) were not different from the control during the calcitonin infusion of 6–20h in this system, but were significantly decreased by the calcitonin infusion for 30h ($P < 0.05$).

The effect of graded doses of calcitonin continuously infused into vitamin D-deficient thyroparathyroidectomized rats is shown in Fig. 3. When the thyroparathyroidectomized rats were infused for 30h with graded amounts of salmon calcitonin, a significant and dose-related increase in the accumulation of 1 α ,25-(OH)₂D₃ was observed (Fig. 3a). Plasma calcium concentrations decreased with continuous infusion of higher concentrations (20 and 500m-units/h) of calcitonin. Plasma concentrations of P_i did not change significantly with infusion of 3–500m-units of calcitonin/h in this system (Fig. 3b).

To verify that the effect of calcitonin on 1 α ,25-(OH)₂D₃ synthesis is not due to contamination in the natural salmon calcitonin preparation used, an independent set of experiments was performed with synthetic salmon calcitonin. The synthetic salmon calcitonin at 500m-units/h for 30h caused a significant stimulatory effect [33.2 ± 1.0 pmol/100ml (mean $\pm$ S.E., $n = 4$), compared with 16.8 ± 3.8 pmol/100ml (mean $\pm$ S.E., $n = 4$) in the control ($P < 0.01$).

Table 1 shows the comparative effects of salmon calcitonin, bovine parathyrin and cyclic AMP on the accumulation *in vivo* of 1 α ,25-(OH)₂[³H]D₃ in vitamin D-deficient thyroparathyroidectomized rats infused with 5mM-CaCl₂ for 30h. Infusion of 7.5 units of parathyrin/h or of 2 μ mol of cyclic AMP/h (maximal doses of parathyrin and cyclic AMP for stimulating 1 α -hydroxylation reaction; see Horiuchi *et al.*, 1977) for 30h into the vitamin D-deficient thyroparathyroidectomized rats caused a marked increase in plasma concentrations of 1 α ,25-(OH)₂[³H]D₃ as reported previously (Horiuchi *et al.*, 1977). When 500m-units of calcitonin/h was

Fig. 3. Dose-response relation of the calcitonin effect on the accumulation *in vivo* of 1 α ,25-(OH)₂[³H]D₃ (a), and on the plasma concentrations (b) of calcium and phosphorus. Experiments were performed as in Fig. 2 except that rats were infused with graded amounts of calcitonin for 30h immediately after thyroparathyroidectomy. Symbols: ○, plasma calcium; □, plasma phosphorus. The vertical bar represents the S.E.M., and the numbers in parentheses indicate the numbers of animals. Results that are significantly different from the control values are indicated as follows: * $P < 0.05$; † $P < 0.001$.

infused concomitantly with 7.5 units of parathyrin/h, the co-operative stimulatory effect of the respective hormone was observed. Concurrent infusion of 7.5 units of parathyrin/h with 2 μ mol of cyclic AMP/h did not cause further enhancement of the concentration obtained by either parathyrin or cyclic AMP.

The plasma calcium concentration of the rats infused with cyclic AMP was almost identical with that of the control (Table 1). The plasma concentration of calcium in the thyroparathyroidectomized rats increased markedly with constant infusion of 7.5 units of parathyrin/h. The concomitant infusion of calcitonin (500m-units/h) with parathyrin significantly suppressed the hypercalcaemic action of parathyrin. The plasma P_i concentrations were not different among the control, parathyrin-treated and parathyrin-plus-calcitonin-treated groups.

Table 1. *The comparative effects of parathyrin, calcitonin and cyclic AMP individually or in combinations on the in vivo accumulation of 1 α ,25(OH) $_2$ [3 H]D $_3$ in vitamin D-deficient thyroparathyroidectomized rats infused with 5 mM-CaCl $_2$ for 30 h*
Data are means $\pm$ S.E.M. All chemicals were infused immediately after thyroparathyroidectomy. After 24 h, the rats were given 25(OH)[3 H]D $_3$ and the plasma was collected 6 h thereafter. Results that are significantly different from the parathyrin (7.5 units/h)-treated group are indicated as follows: * P < 0.05; † P < 0.01; ‡ P < 0.001.

Chemicals infused	Number of rats	1 α ,25(OH) $_2$ [3 H]D $_3$ recovered in plasma (pmol/100 ml)	Plasma concentration 30h after thyroparathyroidectomy of:	
			Calcium (mg/100ml)	Phosphorus(mg/100ml)
Control	9	11.4 $\pm$ 1.0‡	5.6 $\pm$ 0.4‡	9.3 $\pm$ 0.6
Parathyrin (7.5 units/h)	4	28.3 $\pm$ 2.0	11.1 $\pm$ 0.8	9.3 $\pm$ 0.7
Parathyrin (7.5 units/h) and calcitonin (500 m-units/h)	4	47.9 $\pm$ 2.8†	7.3 $\pm$ 0.8*	8.2 $\pm$ 1.2
Cyclic AMP (2 μ mol/h)	5	29.7 $\pm$ 1.3	5.8 $\pm$ 0.2‡	6.1 $\pm$ 0.3†
Parathyrin (7.5 units/h) and cyclic AMP (2 μ mol/h)	5	24.1 $\pm$ 1.9	13.2 $\pm$ 0.7	7.0 $\pm$ 0.4*

Table 2. *Comparative effects of calcitonin and/or cyclic AMP on the in vivo accumulation of 1 α ,25(OH) $_2$ [3 H]D $_3$ in vitamin D-deficient thyroparathyroidectomized rats infused with 10 mM-CaCl $_2$ for 30 h*

Data are means $\pm$ S.E.M. All chemicals were infused immediately after thyroparathyroidectomy. After 24 h, the rats were given 25(OH)[3 H]D $_3$ and the plasma was collected 6 h thereafter. Results that are significantly different from the calcitonin (500 m-units/h)-treated group are indicated as follows: * P < 0.05; ‡ P < 0.001.

Chemicals infused	Number of rats	1 α ,25(OH) $_2$ [3 H]D $_3$ recovered in plasma (pmol/100 ml)	Plasma concentration 30h after thyroparathyroidectomy of:	
			Calcium (mg/100ml)	Phosphorus(mg/100ml)
Control	10	15.2 $\pm$ 1.7‡	6.5 $\pm$ 0.3*	7.6 $\pm$ 0.6
Calcitonin (500 m-units/h)	6	25.9 $\pm$ 1.3	5.3 $\pm$ 0.5	8.9 $\pm$ 0.9
Cyclic AMP (2 μ mol/h)	4	33.2 $\pm$ 2.7*	6.5 $\pm$ 0.5	6.3 $\pm$ 0.8
Cyclic AMP (2 μ mol/h) and calcitonin (500 m-units/h)	5	46.1 $\pm$ 2.7‡	7.7 $\pm$ 0.7*	5.8 $\pm$ 0.9*

Table 3. *Failure of theophylline to potentiate the effects of submaximal doses of calcitonin on the in vivo accumulation of 1 α ,25(OH) $_2$ [3 H]D $_3$ in vitamin D-deficient thyroparathyroidectomized rats infused with 10 mM-CaCl $_2$ for 30 h*

Data are means $\pm$ S.E.M. All chemicals were infused immediately after thyroparathyroidectomy. After 24 h, the rats were given 25(OH)[3 H]D $_3$ and the plasma was collected 6 h thereafter.

Chemicals infused	Number of rats	1 α ,25(OH) $_2$ [3 H]D $_3$ recovered in plasma (pmol/100 ml)	Plasma concentration 30h after thyroparathyroidectomy of:	
			Calcium (mg/100ml)	Phosphorus(mg/100ml)
Control	10	15.2 $\pm$ 1.7	6.5 $\pm$ 0.3	7.6 $\pm$ 0.6
Theophylline (0.5 μ mol/h)	5	17.4 $\pm$ 1.2	6.3 $\pm$ 0.3	9.2 $\pm$ 0.8
Calcitonin (3 m-units/h)	6	17.0 $\pm$ 2.7	6.8 $\pm$ 0.4	8.5 $\pm$ 0.5
Calcitonin (3 m-units/h) and theophylline (0.5 μ mol/h)	4	13.3 $\pm$ 2.3	6.1 $\pm$ 0.3	7.5 $\pm$ 0.7
Calcitonin (20 m-units/h)	6	22.3 $\pm$ 2.3	5.2 $\pm$ 0.4	7.6 $\pm$ 0.7
Calcitonin (20 m-units/h) and theophylline (0.5 μ mol/h)	4	23.5 $\pm$ 2.6	5.0 $\pm$ 0.2	9.9 $\pm$ 0.5

Table 2 indicates the comparative effects of salmon calcitonin and cyclic AMP on the accumulation *in vivo* of 1 α ,25(OH) $_2$ D $_3$, where vitamin D-deficient thyroparathyroidectomized rats were infused with 10 mM-CaCl $_2$ for 30 h. Infusion of a maximal

dose (500 m-units/h) of salmon calcitonin or 2 μ mol of cyclic AMP/h markedly increased plasma concentrations of 1 α ,25(OH) $_2$ [3 H]D $_3$. The concurrent infusion of 500 m-units of salmon calcitonin/h with 2 μ mol of cyclic AMP/h was additive to the stimula-

tory effect of either calcitonin or cyclic AMP on the concentrations of $1\alpha,25(\text{OH})_2[^3\text{H}]\text{D}_3$ (Table 2). The concurrent infusion of theophylline did not potentiate the effects of submaximal doses (3 and 20 m-units/h) of salmon calcitonin (Table 3).

Discussion

Experimental results showing that calcitonin stimulates the *in vivo* conversion of $25(\text{OH})\text{D}_3$ into $1\alpha,25(\text{OH})_2\text{D}_3$ have been reported by Galante *et al.* (1972) and Lorenc *et al.* (1977). Both groups employed vitamin D-deficient rats with an intact thyro-parathyroid system. Lorenc *et al.* (1977) demonstrated that subcutaneous administration of 270 m-units of calcitonin every 8 h for 2 days increased the accumulation of $1\alpha,25(\text{OH})_2[^3\text{H}]\text{D}_3$ from $25(\text{OH})[^3\text{H}]\text{D}_3$ in plasma and intestine, and that the calcitonin effect disappeared when parathyroid glands were removed. These results led them to conclude that the stimulatory effect of calcitonin on $1\alpha,25(\text{OH})_2\text{D}_3$ synthesis is mainly due to a secondary response of parathyroid glands (Lorenc *et al.*, 1977).

The present study, on the other hand, clearly indicates that continuous infusion of 20 m-units of calcitonin/h into vitamin D-deficient thyro-parathyroidectomized rats enhances the *in vivo* accumulation of $1\alpha,25(\text{OH})_2[^3\text{H}]\text{D}_3$ from $25(\text{OH})[^3\text{H}]\text{D}_3$. The dose used in the present study appears comparable with that used by Lorenc *et al.* (1977). The cause(s) of the discrepancy between our findings and those of Lorenc *et al.* (1977) is not clear at the present time, but may be due to differences in experimental conditions. Lorenc *et al.* (1977) administered synthetic salmon calcitonin subcutaneously every 8 h for 2 days into thyro-parathyroidectomized rats fed a vitamin D-deficient diet containing 1.2% calcium and 0.1% phosphorus, whereas we infused calcitonin continuously for 30 h into thyro-parathyroidectomized rats fed a vitamin D-deficient diet containing 0.45% calcium and 0.3% phosphorus. The calcitonin effect on $1\alpha,25(\text{OH})_2\text{D}_3$ synthesis is probably not due to contamination of the calcitonin preparation used, because the effect was reproduced by a synthetic specimen of salmon calcitonin.

It is noteworthy that in the present experimental conditions the stimulation by calcitonin of $1\alpha,25(\text{OH})_2\text{D}_3$ synthesis is independent of the stimulatory effect of parathyrin. Concomitant infusion of calcitonin with parathyrin, both at maximal doses, had an additive effect on the accumulation of $1\alpha,25(\text{OH})_2\text{D}_3$. We have reported that the stimulatory effect of parathyrin on biosynthesis of $1\alpha,25(\text{OH})_2\text{D}_3$ *in vivo* is mediated by cyclic AMP (Horiuchi *et al.*, 1977). That the calcitonin effect was not potentiated by infusion of theophylline and was additive to that of cyclic AMP also supports the concept that the calcitonin effect is not mediated by cyclic AMP.

Serum concentrations of P_i , another potential factor for the control of $1\alpha,25(\text{OH})_2\text{D}_3$ production, remained unchanged during the course of calcitonin action in the present experimental condition. However, P_i concentrations in renal tubules may have been increased as a consequence of calcitonin action on the kidney, since Talmage *et al.* (1972) have suggested that calcitonin increases intracellular P_i concentrations. Russell (1976), on the other hand, has suggested that calcitonin may increase P_i excretion by the kidney, which might result in a decreased P_i concentration within renal cells. There is, however, little direct evidence regarding changes in P_i concentration within renal cells and within intracellular compartments in response to calcitonin. Calcitonin is also believed to induce a change in the intracellular distribution of calcium in the kidney (Borle, 1975). It is worthwhile also to consider whether mitochondrial calcium may be involved in stimulating $1\alpha,25(\text{OH})_2\text{D}_3$ synthesis by calcitonin. Calcitonin enhances the uptake of calcium into mitochondria (Borle, 1975), and mitochondrial calcium appears to be closely related to renal 1α -hydroxylase activity (Horiuchi *et al.*, 1975; Bikle *et al.*, 1975; Bikle & Rasmussen, 1975). In experiments *in vitro*, the interaction of these ions has been shown to cause a modulation of the mitochondrial 1α -hydroxylase activity (Bikle *et al.*, 1975; Bikle & Rasmussen, 1975). Therefore, these mechanisms could be a means by which calcitonin causes a stimulation of the 1α -hydroxylation reaction in the kidney.

The biological significance of the stimulatory effect of calcitonin on $1\alpha,25(\text{OH})_2\text{D}_3$ synthesis is not known. More recently, attention has been focused on calcium metabolism in lactating animals. Plasma concentrations of $1\alpha,25(\text{OH})_2\text{D}_3$ (Boass *et al.*, 1977) and calcitonin (Toverud *et al.*, 1978) have been reported to be significantly higher in lactating than in non-lactating rats. In suckling rats (Cooper *et al.*, 1977) and in young rats (Talmage *et al.*, 1975), plasma calcitonin concentrations tend to be increased. These animals are all in a state of highly positive calcium balance. Thus it appears possible that calcitonin may be involved in the stimulation of renal biosynthesis of $1\alpha,25(\text{OH})_2\text{D}_3$ in these animals. Preliminary experiments demonstrated that bovine prolactin failed to enhance $1\alpha,25(\text{OH})_2\text{D}_3$ production in the present infusion system. Therefore, increased plasma concentrations of $1\alpha,25(\text{OH})_2\text{D}_3$ in lactating animals could be explained at least in part by enhanced secretion of calcitonin. It has been reported that plasma calcium concentrations are approx. 1 mg/100 ml lower in lactating rats than in non-lactating rats (Toverud *et al.*, 1978), suggesting that the enhanced secretion of calcitonin decreases the plasma calcium concentrations to counteract negative calcium balance. The stimulation by calcitonin of

renal biosynthesis of 1 α ,25(OH) $_2$ D $_3$ and the consequent enhancement of the intestinal absorption of calcium may reinforce this defence mechanism. In any event, the effect of calcitonin on vitamin D metabolism may give an important clue to the elucidation of calcium homeostasis in growing animals.

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