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PITUITARY 1,25-DIHYDROXYVITAMIN D_3 RECEPTORS IN HYPERTHYROID-AND HYPOTHYROID-RATS

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The binding of 1α,25-dihydroxy(26,27-methyl-[^{3}H])cholecalciferol ([^{3}H]1,25-$(OH)_2D_3$) to its receptor in cytosol of the anterior pituitary cells was examined in hyperthyroid-and hypothyroid rats, as well as in normal rats. The binding capacity increased by 41 % in L-Thyroxine-treated hyperthyroid rats and decreased by 49 % in propylthiouracil-ingested hypothyroid rats as compared with normal control rats, whereas the affinity of the receptor for [^{3}H]-$1,25(OH)_2D_3$ showed no difference among these 3 animal groups.

These findings indicate that the number of $1,25(OH)_2D_3$ receptors in the pituitary may be regulated by thyroid hormone, and further suggest that 1,25-$(OH)_2D_3$ may play some role in regulating functions of the anterior pituitary.

Receptors for $1,25(OH)_2D_3$ have been found not only in the classical target organs of vitamin D, such as the intestine, bone and kidney, but also in other organs, including the pituitary, pancreas, parathyroid, skin, and placenta (1,2,3). Among these newly identified target organs for $1,25(OH)_2D_3$, the pituitary gland is of great interest, since a few reports have demonstrated a regulatory effect of $1,25(OH)_2D_3$ on GH and prolactin secretion from rat pituitary tumor cell lines, though their results are somewhat discrepant (4,5,6). Holick et al. have recently reported a stimulatory effect of $1,25(OH)_2D_3$ on the base-line secretion of TSH, but not on prolactin, from cultured normal rat anterior pituitary cells (7). Furthermore, Sar et al. (8) have demonstrated, using an autoradiographic technique, that $1,25(OH)_2D_3$ receptors may exist in thyrotrophs of the rat pituitary gland.

In order to know whether pituitary receptors for $1,25(OH)_2D_3$ alter according to the status of thyroid function, binding of [^{3}H]$1,25(OH)_2D_3$ to the pituitary cytosol receptors was examined in hyperthyroid and hypothyroid rats, and compared with that in normal control rats.

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MATERIALS AND METHODS

Animals: Six-week-old male Sprague-Dawley rats were obtained from Japan Crea Inc. (Osaka, Japan) and divided into three groups (8-9 rats for each group). The rats of the first group received daily s.c. injections of 50 μg L-thyroxine (T_4) (Sigma Chemical Co., St. Louis) for 2 weeks. The second group was given drinking water containing 0.05 % 6-propyl-2-thiouracil (PTU) (Nakarai Chemical Co., Kyoto, Japan) for 2 weeks *ad libitum*. The last group of animals, which were injected s.c. with the vehicle (NaOH-Saline mixture) alone, served as a control. At the end of the treatment period the animals in each group were weighed, decapitated, and blood samples and pituitary tissues were obtained. Plasma TSH, prolactin and T_4 were measured by radioimmunoassay, as described previously (9,10). Serum calcium was determined using Calcett (Precision Systems, Sudbury, MA).

Materials: 1α,25-dihydroxy-[26,27-methyl-^{3}H]cholecalciferol ([^{3}H]1,25(OH)$_2$D$_3$; SA;158 Ci/mmol) and 25-hydroxy-[23,24-(N)-^{3}H]cholecalciferol ([^{3}H]25OHD$_3$; SA; 158 Ci/mmol) were purchased from Amersham Japan (Tokyo, Japan); nonradioactive vitamin D$_3$ metabolites were gifts from Chugai Pharmaceutical Co. (Tokyo, Japan).

Cytosol preparation: An anterior lobe of the pituitary was carefully separated from the neurointermediate lobe, and finely minced with two razer blades. All subsequent procedures were performed at 4°C. Minced pituitary tissues were washed 3 times with 0.01 M Tris-HCl (pH 7.4), 0.3 M KCl, 1.5 mM EGTA, 5mM dithiothreitol, 10 mM sodium molybdate, and 200 kallikrein-inhibiting units/ml Aprotinine (Buffer A) and homogenized in 5 vol of this buffer using a Teflon-glass homogenizer (10-12 strokes). Cytosol preparations were obtained by centrifuging the homogenates at 80000 x g for 60 min in the 50-Ti rotor of a Beckman L5-50B ultracentrifuge (Beckman Instruments, Palo Alto, CA). Several parts of the brain were also dissected in some experiments, and the binding of 1,25(OH)$_2$D$_3$ to the neural tissue preparations was examined. The protein concentration was determined by the method of Lowry *et al* (11), using bovine serum albumin as the standard.

1,25(OH)$_2$D$_3$ receptor assay: The concentration of 1,25(OH)$_2$D$_3$ receptor was determined by saturation analysis as described previously (12). Briefly, 200 μl aliquots of cytosol were incubated for 2 h at 25°C in duplicate with increasing amounts of [^{3}H]1,25(OH)$_2$D$_3$ (0.01-0.09 pmol) in the presence or absence of a 100-fold excess of nonradioactive hormone. No significant degradation of the receptor occurred during incubation at this temperature. A similar excess of 25OHD$_3$ was used in parallel incubations to ensure specificity of displacement for 1,25(OH)$_2$D$_3$. The free hormone was pelleted with dextran-coated charcoal, and bound radioactivity was determined by a liquid scintillation counter using Univer-Gel II® (Nakarai Chemical Co., Kyoto, Japan) as the scintillation cocktail.

Discontinuous 5-20 % sucrose gradients (w/v, in buffer A) were prepared, allowed to equilibrate at room temperature and then cooled before use. Aliquots of cytosol (200 μl) were incubated for 2 h at 25°c with 0.05 pmol [^{3}H]1,25(OH)$_2$D$_3$ or [^{3}H]25OHD$_3$ in the absence or presence of 5 pmol nonradioactive 1,25(OH)$_2$D$_3$ or 25OHD$_3$. After charcoal adsorption of the unbound steroid, 200 μl aliquots of labeled cytosol were layered onto the top of each gradient, and tubes were centrifuged at 255,000 x g for 20 h at 4°C in the SW-40 rotor of a Beckman L5-50B ultracentrifuge. Pooled eel (A. Anguilla) liver cytosol, labeled by [^{3}H]1,25(OH)$_2$D$_3$ and run in parallel gradients, was used as a sedimentation marker (12).

RESULTS

Pituitary cytosol receptors for 1,25(OH)$_2$D$_3$: Significant binding of [^{3}H]-1,25(OH)$_2$D$_3$, which was quite displaceable by a 100-fold excess of radioinert

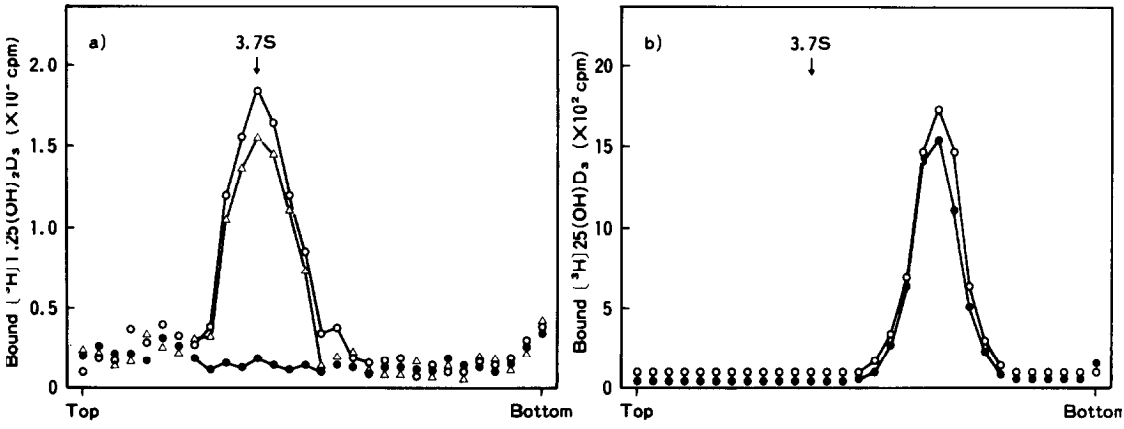

Figure 1.
a.) Sucrose density gradient analysis of $[^3H]1,25(OH)_2D_3$ binding in rat anterior pituitary cytosol. o———o ; $[^3H]1,25(OH)_2D_3$ alone
●———● ; $[^3H]1,25(OH)_2D_3$ + 100-fold excess unlabelled $1,25(OH)_2D_3$
△———△ ; $[^3H]1,25(OH)_2D_3$ + 100-fold excess unlabelled $25OHD_3$
b.) Sucrose density gradient analysis of $[^3H]25OHD_3$ binding in rat anterior pituitary cytosol.
o———o ; $[^3H]25OHD_3$ alone, ●———● ; $[^3H]25OHD_3$ + 100-fold excess unlabelled $25OHD_3$. The arrow indicates the position of eel liver cytosol $1,25(OH)_2D_3$ receptor with a sedimentation coefficient of 3.7 S.

$1,25(OH)_2D_3$, was found using cytosols prepared from pituitary tissues. Non-radioactive $25OHD_3$ in a 100-fold excess failed to inhibit the binding of $[^3H]$-$1,25(OH)_2D_3$ with cytosol receptors of the pituitary. No specific binding was seen in the cerebral cortex, thalamus, hypothalamus, cerebellum, pineal body and spinal cord (data not shown). The specific binding of $[^3H]1,25(OH)_2D_3$ to rat anterior pituitary tissues was also demonstrated by sucrose gradient centrifugation in hypertonic buffer. Representative results are shown in Fig. 1. A single peak of radioactivity with a sedimentation coefficient of 3.7 S was recognized, which was totally abolished by coincubation with a 100-fold excess of $1,25(OH)_2D_3$, but not with $25OHD_3$ (Fig. 1a). When cytosol was similarly incubated with $[^3H]25OHD_3$, another single peak of radioactivity was seen at the position of a sedimentation coefficient larger than 3.7 S. This peak was only slightly reducible by coincubation with an excess of $25OHD_3$ (Fig. 1b).

Hormonal data: Body weight was significantly ($p<0.001$) higher in control rats (331.8±8.1 g) than in PTU-treated rats (275.6±3.9 g), though body weight of

Table 1. Plasma T_4, prolactin, TSH and serum Ca levels in control, T_4-treated and PTU-treated rats

		T_4 (μg/dl)	TSH (μg/dl)	Prolactin (ng/ml)	Ca (mg/dl)
Control	(8)	4.0±0.2	6.0±2.4	26.3±3.5	9.9±0.4
T_4-treated	(8)	9.5±0.5*	n.d.*	33.4±4.1	9.7±0.4
PTU-treated	(8)	n.d.*	479.3±42.2*	22.1±1.6	9.4±0.3

Values are expressed as mean ± SE of the indicated number of rats. n.d.; not detectable, * ; statistical difference (vs control): $p<0.001$ by unpaired Student's t tests.

T_4-treated rats (320.0±6.0 g) showed no difference compared with that of control rats. There were no difference in weights of the pituitaries among these 3 groups. Plasma T_4 concentrations were significantly increased in T_4-treated rats (9.5±0.5 μg/dl) and decreased in PTU-treated rats (not detectable) as compared with those in control rats (4.0±0.2 μg/dl). Plasma TSH levels were decreased (not detectable) in T_4-treated rats and increased (479.3±42.2 μg/dl) in PTU-treated rats as compared with those in control rats (6.0±2.4 μg/dl). Neither plasma prolactin nor Ca levels showed any difference among the 3 groups (Table 1).

Saturation analyses: The incubation study of rat anterior pituitary cytosols with increasing amounts of [^{3}H]1,25(OH)$_2$D$_3$ demonstrated the presence of binding sites saturable by low concentrations of hormone in all 3 groups. Scatchard analysis of these data revealed a straight line, indicating a single class of binding sites. Values of the equilibrium dissociation constants (Kd), determined from the slope of the regression line, were nearly identical (3.4-3.6 x 10^{-11} M) among the 3 groups. On the other hand, the concentration of binding sites was clearly incresed in T_4-treated rats (19.7 fmol/mg protein, 41 % increase) and, reversely, decreased in PTU-treated rats (6.9 fmol/mg protein, 51 % decrease) as compared with that in contral rats (14.0 fmol/mg protein), as shown in Fig. 2. Similar results were obtained in 3 repeated experiments.

DISCUSSION

In this study, we have confirmed the presence of saturable and specific binding proteins for 1,25(OH)$_2$D$_3$ in the rat pituitary gland, which has been

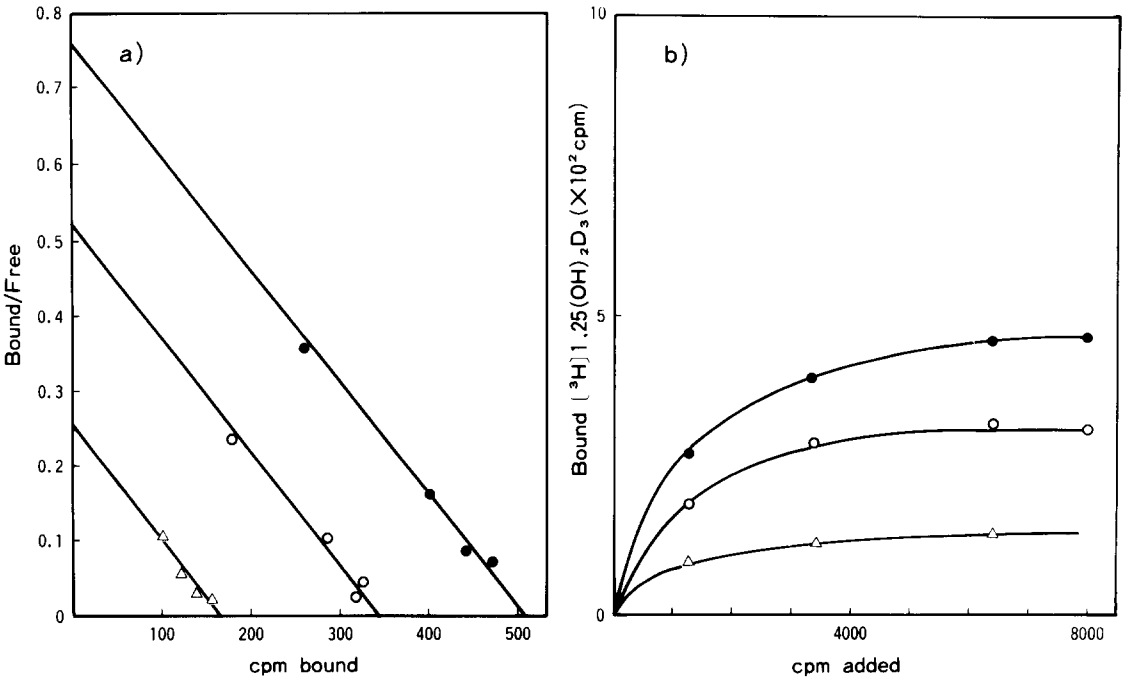

Figure 2.
Scatchard analysis (a) and saturation analysis (b) of specific binding of [^{3}H]1,25(OH)$_2$D$_3$ to the anterior pituitary cytosol of control (o), T_4-treated (●) and PTU-treated rats (△).

previously reported by other laboratories (1,2). In agreement with the findings by Haussler et al, the binding proteins of the pituitary for 1,25-(OH)$_2$D$_3$ possessed low capacity for high-affinity binding, and showed a single peak of 3.7 S in a 5-20 % sucrose gradient analysis, results which are compatible with the biological properties of 1,25(OH)$_2$D$_3$ receptors as reported to exist in other cells and organs (3).

Furthermore, we have demonstrated for the first time that the concentration of pituitary 1,25(OH)$_2$D$_3$ receptors depends on thyroid function: binding of 1,25(OH)$_2$D$_3$ to the receptors was increased in T_4-treated hyperthyroid rats and, conversely, decreased in PTU-ingested hypothyroid rats. The question as to which cells of the anterior pituitary contain the receptors for 1,25-(OH)$_2$D$_3$ is of great importance in considering the physiological roles of 1,25-(OH)$_2$D$_3$ and its receptor at the anterior pituitary. Although at present the question remains unclarified, our present findings showing regulation of pituitary 1,25(OH)$_2$D$_3$ receptors by thyroid hormone could be significant, since

the status of thyroid function is known to influence the synthesis and/or release of anterior pituitary hormones, particularly TSH, prolactin and growth hormone (13-15). Indeed, the presence of $1,25(OH)_2D_3$ receptors in thyrotrophs has been demonstrated by autoradiographic and immunohistochemical techniques (8). Although $1,25(OH)_2D_3$ and its receptor may be closely related with pituitary thyrotrophs, the presence of these functioning receptors in other cells of the anterior pituitary, particularly in lactotrophs and somatotrophs, cannot be ruled out.

The mechanism by which the number of pituitary receptors for $1,25(OH)_2D_3$ alters according to thyroid function remains unknown. In general, little is known about the substances regulating the $1,25(OH)_2D_3$ receptor, except for glucocorticoids, which have been reported to affect the concentration of $1,25-(OH)_2D_3$ receptors in the intestine and bone of rodents (16-18). It is possible that T_4, TSH and other substances which change with thyroid function may all be responsible for modulating the $1,25(OH)_2D_3$ receptor concentration through direct action at the pituitary. Alternatively, the concentration of pituitary $1,25(OH)_2D_3$ receptors may be controlled by a circulating endogenous $1,25(OH)_2D_3$ level according to a rule of down-regulation, since the serum $1,25(OH)_2D_3$ level has been reported to decrease in patients with hyperthyroidism and to increase in hypothyroid subjects (19-21).

REFERENCES

1. Haussler, M.R., Manolagas, S.C. and Deftos, L.J. (1980) J. Biol. Chem. 255, 5007-5010.
2. Pike, J.W., Gooze, L. and Haussler, M.R. (1980) Life Sci. 26, 407-414.
3. Norman, A.W., Roth, J. and Orci, L. (1982) Endocrine Reviews 3, 331-366.
4. Murdoch, G.H. and Rosenfeld, M.G. (1981) J. Biol. Chem. 256, 4050-4055.
5. Haug, E., Pederson, J.I. and Gautvik, K.M. (1982) Mol. and Cell. Endocrinology 28, 65-79.
6. Wark, J.D. and Tashjian, Jr.A.H. (1982) Endocrinology 111, 1755-1757.
7. Holick, M.F. and Rose, S.D. (1985) 6th. Workshop on Vitamin D pp 89. (Abstract)
8. Sar, M., Stumpf, W.E. and Deluca, H.F. (1980) Cell Tissue Res. 209, 161-166.
9. Chihara, K., Kato, Y., Ohgo, S., Iwasaki, Y., Maeda, K., Miyamoto, Y. and Imura, H. (1976) Endocrinology 98, 1396-1400.
10. Chihara, K., Arimura, A., Coy, D.H. and Schally, A.V. (1978) Endocrinology 102, 281-290.Endocrinology
11. Lowry,O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.

12. Marcocci, C., Freake, H.C., Iwasaki, J., Ropez, E. and MacIntyre, I. (1982) Endocrinology 110, 1347-1454.
13. Ching, M., Evans, A.B., Evans, E.S., Joseph, S. and Sorrentino, S. (1974) Acta Endocrinol. (Copenh) 75, 221-232.
14. Spira, O., Birkenfeld, A., Gross, J. and Gordon, A. (1979) Acta Endocrinol. (copenh) 92, 489-511.
15. Peake, G.T., Birge, C.A. and Daughaday, W.H. (1973) Endocrinology 92, 487-493.
16. Hirst, M. and Feldman, D. (1982) Endocrinology 111, 1400-1402.
17. Massaro, E.R., Simpson, R.U. and DeLuca, H.F. (1982) J. Biol. Chem. 257, 13736-13739.
18. Chen, T.L., Cone, C.M., Morey-Holton, E. and Feldman, D. (1982) J. Biol. Chem. 257, 13564-13569.
19. Boullion, R., Muls, E. and DeMoor, P. (1980) J. Clin. Endocrinol. Metab. 51, 793-797.
20. Jastrup, B., Mosekilde, L., Melson, F., Lund, Bi., Lund, Bj. and Sorensen, O.H. (1982) Metabolism 31, 126-132.
21. MacFarlane, I.A., Mawer, E.B., Berry, J. and Hann, J. (1982) Clin. Endocrinol. 17, 51-59.