Effect of 1,25-dihydroxyvitamin D₃ on TSH secretion from rat pituitary cells in culture

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Abstract. The effect of 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) on TSH secretion from rat pituitary cells was studied. When incubating cells with 1,25(OH)₂D₃ even at 100 × the physiological concentrations (10⁻⁸), no effect on basal TSH secretion was observed. The TRH-induced TSH secretion increased after a 24-h incubation with 10⁻⁸ mol/l 1,25(OH)₂D₃ (2.9 ± 0.2 ng/well vs 4.3 ± 0.5 ng/well, mean ± sd; P < 0.05). When serum was omitted from the incubation medium, the potentiating effect of 1,25(OH)₂D₃ on the TRH-induced TSH release was blunted. No effect on cellular protein content was observed after incubating the cells with 10⁻⁸ mol/l 1,25(OH)₂D₃. The results indicate that at unphysiological concentrations, 1,25(OH)₂D₃ affects the TRH-induced TSH secretion from pituitary cells. The physiological significance remains unclear.

In recent years, the interaction of the most active vitamin D₃ metabolite, 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), and the pituitary has been subject to increasing interest. There is evidence indicating that GH in rats, and perhaps PRL in hens, participates in the regulation of 1,25(OH)₂D₃ (Spanos et al. 1976; Pahuja & De Luca, 1981). Using isolated rat kidneys, Kano & Jones (1984) showed that TSH decreased the formation of 1,25(OH)₂D₃ in vitro.

Sar et al. (1980) observed that the rat pituitary and especially the thyrotropes bind 1,25(OH)₂D₃. The discovery of receptors for 1,25(OH)₂D₃ both in rat pituitary cells and in PRL and GH secreting rat tumour cells supports this suggestion (Haussler et al. 1981, 1982). Recently we reported that 1,25(OH)₂D₃ enhances the TRH-induced TSH secretion in rats in vivo and in rat pituitary cells in vitro (Törnquist & Lamberg-Allardt 1985, 1986). In this report we further examine the effect of 1,25(OH)₂D₃ on the synthesis and secretion of TSH in normal rat anterior pituitary cells in primary culture.

Materials and Methods

Female Wistar rats (180–220 g) were housed in temperature controlled quarters with a 12-h light-dark cycle, and the rats were fed a standard diet and had tap water ad libitum.

They were sacrificed by CO₂ gassing and cervical dislocation. The pituitary was dissected and the anterior lobe dispersed using 0.3% collagenase (Boehringer, Mannheim) and 3% bovine serum albumin (BSA) in phosphate buffered saline (PBS) for 20–40 min followed by 0.2% deoxyribonuclease (Sigma) for 10 min. The cells were washed five times with PBS, with a 10-min centrifugation at 280 × g between each wash. The dispersed cells were plated on Falcon multi-well plastic dishes at a concentration of 1.5–2.0 × 10⁵ cells/dish in Dulbecco's modified Ear's medium (DMEM) with 10% horse serum, 2.5% calf serum, 1% Gibco non-essential amino acids and penicillin-streptomycin (5 × 10⁴ U/l resp. 50 mg/l). The cells were allowed to attach to the dishes for 2 days in a water saturated atmosphere of 95% air and 5% CO₂ at 37°C, whereafter the medium was changed to DMEM containing 1,25(OH)₂D₃ (Roche OY, Espoo, Finland; concentrations as indicated) in 5 μl ethanol/3.5 ml DMEM (3 wells/concentration). In some experiments, serum was omitted from DMEM. No difference in free calcium was observed in the presence or absence of serum.
Controls received vehicle only. After 24 h, the medium was collected, the cells were washed with 2 × 0.5 ml of PBS, and new medium containing 1,25(OH)$_2$D$_3$ and TRH (Roche OY, Espoo, Finland) was added. After a 0.5–2 h incubation, the medium was collected and the cells were washed with 2 × 0.5 ml of PBS. If the cells were submitted to longer incubations with 1,25(OH)$_2$D$_3$, the medium was changed every 48 h and the last 48-h medium saved for TSH analysis.

In the K$^+$ depolarisation experiments, 50 mmol/l K$^+$ was added to the cells and incubated for 0.5 h. Equimolar concentrations of Na$^+$ was used to control the effect of increased osmolarity.

TSH was measured with a NIADDK rat TSH RIA kit kindly provided by Dr F. Parlow, with NIADDK-rTSH-RP-2 as standard. Both the within- and the between-assay variation were always less than 20%. Protein was determined according to Lowry et al. (1951) with BSA as standard. The data are shown as the mean ± sd. Statistical significance was evaluated using analysis of variance, Scheffe’s test, and Student’s t-test.

Results

No significant effect of 1,25(OH)$_2$D$_3$ on basal TSH release was observed with the concentrations tested after a 24-h incubation (Fig. 1a). The TRH-induced TSH secretion increased significantly in the presence of 10$^{-8}$ mol/l 1,25(OH)$_2$D$_3$, $P < 0.05$ (Fig. 1b). 1,25(OH)$_2$D$_3$ did not affect the total TSH (24-h basal secretion, TRH-induced TSH) (Fig. 1c). When TSH secretion was induced by K$^+$ depolarisation, no effect on TSH secretion was obtained with increasing doses of 1,25(OH)$_2$D$_3$ (Fig. 2). The addition of equimolar concentrations of Na$^+$ did not affect TSH secretion.

Incubating the cells with 10$^{-8}$ mol/l 1,25(OH)$_2$D$_3$ and increasing amounts of TRH showed that the difference in TSH secretion between control cells and 1,25(OH)$_2$D$_3$ treated cells

**Fig. 1.**

Effect of a 24-h incubation of rat pituitary cells with increasing amounts of 1,25(OH)$_2$D$_3$ on a) basal TSH secretion, b) TSH secretion induced by a 2-h incubation with 10$^{-7}$ mol/l of TRH, and c) total TSH secreted (basal and TRH-induced TSH secretion). C = control. (Mean ± sd, N = 2–3, *P < 0.05).

**Fig. 2.**

Effect of a 24-h incubation of rat pituitary cells with increasing amounts of 1,25(OH)$_2$D$_3$ on TSH secretion induced by a 0.5-h incubation with K$^+$ (K$^+$ = 50 mmol/l). C = control. (Mean ± sd, N = 2–3).
increased with increasing amounts of TRH. A significant difference was reached with $10^{-7} \text{ mol/l}$ TRH ($P < 0.01$, Fig. 3). In Fig 4 the effect of prolonged incubation of cells with $10^{-8} \text{ mol/l}$ 1,25(OH)$_2$D$_3$ is shown. Long incubations (> 24 h) did not affect basal TSH release significantly. The enhanced effect of 1,25(OH)$_2$D$_3$ on TRH-induced TSH secretion was also blunted after long incubations (data not shown). Incubating rat pituitary cells with increasing amounts of 1,25(OH)$_2$D$_3$ did not significantly increase cellular protein content.

If both foetal calf serum and horse serum were excluded from the incubation medium, no difference in basal TSH secretion was observed between 1,25(OH)$_2$D$_3$ treated and control cells. The enhancing effect of 1,25(OH)$_2$D$_3$ on TRH-induced TSH secretion was also blunted (Table 1).

Discussion

Reports considering the interaction between 1,25-(OH)$_2$D$_3$ and pituitary hormone secretion are contradictory. Murdoch & Rosenfeld (1981) and Haug et al. (1982) reported that 1,25(OH)$_2$D$_3$ decreased basal PRL secretion in rat GH3 tumour cells, whereas Wark & Tashjian (1982) obtained the opposite effect using rat GH$_4$C$_1$ tumour cells. Recently Rose & Holick (1985) showed that 1,25-(OH)$_2$D$_3$ increased basal TSH secretion in cultured normal rat pituitary cells. We observed that 1,25(OH)$_2$D$_3$ was without any effect on basal TSH levels in rats in vivo (Törnquist & Lamberg-Allardt 1985), but that 1,25(OH)$_2$D$_3$ enhanced the TRH-induced TSH secretion both in rats in vivo and from rat pituitary cells in vitro (Törnquist & Lamberg-Allardt 1985, 1986).

In the present study we found that 1,25(OH)$_2$D$_3$ had no effect on basal TSH secretion which is consistent with our observations in vivo (Törn-
Table 1.
Effect of 1,25(OH)2D3 on TRH-induced TSH secretion in the presence or absence of serum in the incubation medium. The results are from two different experiments. (Mean ± SD; N = 2–3).

<table>
<thead>
<tr>
<th>Concentration of 1,25(OH)2D3 (mol/l)</th>
<th>TRH-induced TSH secretion (ng TSH/well)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>With serum</td>
</tr>
<tr>
<td>Control</td>
<td>2.9 ± 0.2</td>
</tr>
<tr>
<td>10^-10</td>
<td>3.9 ± 0.3</td>
</tr>
<tr>
<td>10^-9</td>
<td>4.2 ± 0.3</td>
</tr>
<tr>
<td>10^-8</td>
<td>4.3 ± 0.5*</td>
</tr>
</tbody>
</table>

* P < 0.05 within one experiment.

The physiological significance of our observation is still an open question. We observed that only at unphysiological concentrations did 1,25-(OH)2D3 affect the TRH-induced TSH secretion. This indicates a pharmacological effect of 1,25-(OH)2D3. Because TSH, T3, and T4 may decrease the synthesis of 1,25(OH)2D3 (Weisman et al. 1981; Kano & Jones 1984; Kano 1984) our results, though, possibly indicate the existence of a feed-back loop between the vitamin D homeostasis and the anterior pituitary hormones. Further studies, however, are needed to clarify our observations.

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References


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