VITAMIN D₃ EFFECT ON THE CALCIUM CONTENT IN SUBCELLULAR FRACTIONS OF RAT INTESTINAL MUCOSA

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IITOYO et al. and LAWSON et al. reported that vitamin D₃ caused a slight decrease in the activities of Mg⁺⁺-activated DNA-dependent RNA polymerase (I, 2) and Mn⁺⁺-activated DNA-dependent RNA polymerase (2). However, it is known that calcium inhibits RNA polymerase (3). Since the calcium content of the intestine in vitamin D-treated animals must be higher, the decrease in RNA polymerase activities by vitamin D₃ might be considered to be derived from the increases in nuclear calcium. Gounelle and Raoul demonstrated that the calcium content in rat liver nuclei was slightly increased by vitamin D₃ (4). However, there is no information about the calcium content in the subcellular fractions of rat intestinal mucosa. Thus, in this report, calcium content in the subcellular fractions of rat intestinal mucosa with various vitamin D₃ status was determined by atomic absorption spectroscopy, and the effect of vitamin D₃ on RNA polymerase activities was discussed.

Vitamin D-deficient rats and their intestinal mucosal subcellular fractions were prepared as described by IITOYO et al. (I). Female rats of Wistar strain weighing 40–50 g were raised on Rachitogenic diet No. 2, U.S.P. (N.B.Co.) for 4 weeks in a dark room. Vitamin D₃ was dissolved in the mixture of ethanol and propylene-glycol (1:9) and a part of the solution equivalent to 2,000 IU of vitamin D₃ was orally administered to vitamin D-deficient rats 1, 3 and 16 hr before sacrifice. Animals were fasted 24 hr before sacrifice. The calcium contents of rat intestinal mucosal subcellular fractions were measured by the method of atomic absorption spectroscopy after ashing of cellular fractions (5).

As shown in Table 1, the calcium content in homogenate of intestinal mucosa was not significantly changed by vitamin D₃ administration, whereas the subcellular distribution of calcium was changed significantly. The intestinal mitochondria the calcium in vitamin D-deficient rat was high, and it was decreased significantly.

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Table 1. Vitamin D$_3$ effect on the calcium content in the subcellular fractions of rat intestinal mucosa. Each value is expressed as $\mu$g calcium/mg protein ± S.E.M. of 5 determinations.

<table>
<thead>
<tr>
<th>Vitamin D status</th>
<th>Homogenate</th>
<th>Mitochondria</th>
<th>Microsomes</th>
<th>Nuclei</th>
<th>Supernatant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D$_3$ deficient</td>
<td>3.014±0.348</td>
<td>7.274±0.911</td>
<td>2.783±0.602</td>
<td>2.776±0.067</td>
<td>1.161±0.018</td>
</tr>
<tr>
<td>fed 1 hr</td>
<td>3.334±0.308</td>
<td>6.226±0.710</td>
<td>5.563±0.427$^b$</td>
<td>2.329±0.444</td>
<td>1.128±0.106</td>
</tr>
<tr>
<td>3 hr</td>
<td>3.020±0.275</td>
<td>3.856±0.466$^e$</td>
<td>5.584±0.559$^b$</td>
<td>2.759±0.269</td>
<td>1.488±0.048$^a$</td>
</tr>
<tr>
<td>16 hr</td>
<td>2.624±0.151</td>
<td>3.924±0.334$^d$</td>
<td>4.348±0.184$^d$</td>
<td>3.301±0.187$^d$</td>
<td>1.040±0.042$^d$</td>
</tr>
</tbody>
</table>

a, b, c, d and e: Significantly different from vitamin D deficient at a: $p<0.001$, b: $p<0.005$, c: $p<0.01$, d: $p<0.02$, and e: $p<0.025$.

by vitamin D$_3$. This result coincided with the results that vitamin D$_3$ stimulated the release of calcium from kidney mitochondria in vitro (6-7). In contrast to mitochondrial calcium, microsomal calcium was significantly increased by vitamin D$_3$. The supernatant calcium concentration was lower than that of mitochondria, microsomes and nuclei, and it was not changed with vitamin D status except for 3 hr after vitamin D$_3$ administration. Nuclear calcium was not changed for 3 hr, but it increased 16 hr after vitamin D$_3$ administration. This result is comparable to Mg$^{++}$-activated RNA polymerase activity changes with vitamin D$_3$ (1). Thus, the increase of nuclear calcium by vitamin D$_3$ administration would in turn decrease RNA polymerase activity.

Although the relation between vitamin D$_3$-induced reduction of RNA polymerase activity and the physiological function of vitamin D$_3$ is presently unclear, it is remarkable that vitamin D$_3$ acts on the subcellular distribution of calcium and controls cellular activity in a manner similar to that of RNA polymerase.

REFERENCES

7) ENGSTROM, G. W. and DELUCA, H. F., Biochemistry, 3, 203 (1964),