SHORT COMMUNICATION

Stimulation of calcium absorption and apparent increased intestinal 1,25-dihydroxycholecalciferol in rats treated with low doses of ethane-1-hydroxy-1,1-diphosphonate

D. GUILLAND, U. TRECHSEL, J.-P. BONJOUR AND H. FLEISCH

Department of Pathophysiology, University of Berne, Berne, Switzerland

(Received 8 November 1974)

Summary

1. Ligated intestinal segments from rats treated with disodium ethane-1-hydroxy-1,1-diphosphonate (EHDP) at the daily dose of 16 μmol (= 1 mg of phosphorus)/kg subcutaneously for 7 days show an increased rate of calcium absorption.

2. This dose of EHDP enhances the intestinal accumulation of a vitamin D₃ metabolite with the chromatographic characteristics of 1,25-dihydroxycholecalciferol.

Key words: disodium ethane-1-hydroxy-1,1-diphosphonate, calcium absorption, 1,25-dihydroxycholecalciferol.

Introduction

The administration of disodium ethane-1-hydroxy-1,1-diphosphonate (EHDP) to rats has been shown to elicit effects on the intestinal absorption of calcium which are directionally different according to the dose employed (Gasser, Morgan, Fleisch & Richelle, 1972). A daily subcutaneous dose of 16 μmol of EHDP/kg (= 1 mg of phosphorus/kg) stimulates, whereas a dose ten times larger markedly depresses calcium absorption (Gasser et al., 1972), this last effect being accompanied by an inhibition of cartilage and bone mineralization (Schenk, Merz, Mühlbauer, Russell & Fleisch, 1973; Gasser et al., 1972). Recently much attention has been given to the large dose of EHDP, and results indicated that the diphosphonate can block the production of 1,25-dihydroxycholecalciferol [1,25-(OH)₂D₃] in rats (Hill, Lumb, Mawer & Stanbury, 1973) and chicks (Baxter, LeLuca, Bonjour & Fleisch, 1974). This vitamin D₃ metabolite is able to prevent or correct the reduced calcium absorption, but not the inhibition of mineralization (Bonjour, DeLuca, Fleisch, Trechsel, Matejovec & Omdahl, 1973a; Bonjour, DeLuca, Baxter, Fleisch & Trechsel, 1973b; Baxter et al., 1974).

In the present study we have investigated whether the stimulation of calcium absorption observed with a low dose of EHDP is also mediated by a change in the metabolism of vitamin D₃. Therefore intestinal calcium transport and metabolism of 25-[³H]-hydroxycholecalciferol has been studied in rats fed with a vitamin D₃ deficient diet after treatment with 16 μmol (1 mg of P) of EHDP/kg daily for 7 days.

Methods

The rats used in this study were Wistar strain from our own breeding maintained on a diet low in vitamin D (Altromin C 1034) with the calcium and phosphate adjusted to 0.5 and 0.3% respectively. The effect of EHDP given at the dose of 16 μmol (1 mg of P)/kg body wt subcutaneously (s.c.) for 7 days on the concentration of plasma calcium and phosphate, intestinal calcium absorption and the width of the epiphyseal plate of tibia was studied on female rats. A control group received 0.15 mol/l NaCl s.c. for 7 days. Blood samples were taken on the seventh day of treatment before the last injection of EHDP. Plasma calcium was determined...
by atomic absorption spectroscopy and plasma inorganic phosphate according to a technique previously described (Bisaz, Russell & Fleisch, 1968). On the eighth day after overnight deprivation of food the capacity of the intestine to absorb calcium was assessed in ligated segments of the duodenum according to a technique already described (Bonjour et al., 1973b), with only one modification: the isotonic solution injected into the intestinal lumen contained $4 \times 10^{-3}$ mol/l instead of $4 \times 10^{-5}$ mol/l CaCl$_2$. At the end of the experiment one entire tibia was dissected out and fixed in aqueous 50% ethanol. Measurement of the width of the epiphyseal plate was performed on three longitudinal sections of the upper part of the tibia, stained with a solution of silver nitrate according to Von Kossa's technique.

The production of vitamin D$_3$ metabolite in vivo was determined in two separate experiments: the first with control and treated groups of eight rats, which were first-generation offspring of rats maintained on the low vitamin D diet mentioned above, and the second with groups of twelve rats, which were second-generation offspring. Starting weights of 100-120 g were used in both experiments. The animals were injected daily for 1 week with either EHDP (16 $\mu$mol of EHDP/kg) or with a 0.15 mol/l NaCl solution. Starting weights of 100-120 g were used in both experiments. The animals were injected daily for 1 week with either EHDP (16 $\mu$mol of EHDP/kg) or with a 0.15 mol/l NaCl solution. At the end of this period 1 nmol of 25-[$\alpha$,27-3H]hydroxycholecalciferol, in 50 $\mu$l of 96% ethanol, was injected intravenously. In the first experiment the amount of radioactivity injected was 5 $\mu$Ci and in the second experiment 3 $\mu$Ci.

The rats were killed by exsanguination 24 h later and the plasma, liver, kidney and small intestine collected and stored frozen at $-20^\circ$C until analysis. In the second experiment only plasma and intestine were taken.

The tissues were extracted and radioactivity was counted as described by Lund & DeLuca (1966) with the modification of Mawer, Backhouse, Holman, Lumb & Stanbury (1972). The chloroform extracts were reduced to dryness under vacuum and taken up in a small amount of chloroform–hexane (13:7, v/v) for chromatography on Sephadex LH-20, according to the system of Holick & DeLuca (1971) with flow rate 0.9 ml/min. The radioactivity counts from the fractions of each peak were added and expressed as percentage of the total radioactivity recovered from the column. The recovery of radioactivity applied to the column ranged from 90 to 95%.

**Results**

Rats treated daily with EHDP at 16 $\mu$mol (1 mg of P)/kg s.c. for 7 days displayed a significantly higher ($P < 0.05$) calcium absorption than untreated control animals. The percentage of $^{45}$Ca absorbed from the intestinal lumen within the 15 min incubation was $45.0 \pm 2.8$ (mean value $\pm$ SEM, $n = 8$) for the EHDP-treated group, versus $35.8 \pm 2.9$ for the control group. The percentage of radioactivity recovered in the intestinal wall of the incubated segment was similar in both groups (EHDP-treated 5.7 $\pm$ 0.5, control 4.4 $\pm$ 0.6), indicating that the transepithelial absorptive process was enhanced by this dose of the diphosphonate. No significant difference was found for plasma calcium (EHDP-treated 2.52 $\pm$ 0.075 mmol/l, control 2.52 $\pm$ 0.05 mmol/l), plasma phosphate (EHDP-treated 2.81 $\pm$ 0.097 mmol/l, control 2.68 $\pm$ 0.097 mmol/l), and the width of the epiphyseal plate of the tibia (EHDP-treated 256 $\mu$m $\pm$ 21, control 231 $\pm$ 16).

The same dose of EHDP which stimulates calcium absorption was shown to bring about an alteration in the radioactivity elution profile of intestinal extracts chromatographed on Sephadex LH-20. As shown in Table 1, the percentage of radioactivity eluted in the position of 1,25-(OH)$_2$D$_3$ was significantly higher in the intestinal extracts of the EHDP-treated rats. This significant difference was detected in both experiments. The amount of the total radioactivity recovered in the intestine (c.p.m./g wet wt.) was not significantly different in EHDP-treated and control rats: Expt. 1: control 7276 $\pm$ 287, EHDP-treated 7146 $\pm$ 479; Expt. 2: control 5872 $\pm$ 813, EHDP-treated 6387 $\pm$ 953 (mean value $\pm$ SEM). This indicates that the increased radioactivity found in the 1,25-(OH)$_2$D$_3$ peak corresponds to an actual rise in the amount of this metabolite in the intestinal tissue. The rise in the 1,25-(OH)$_2$D$_3$ peak radioactivity was accompanied by a decrease of the percentage of 25-hydroxycholecalciferol, which was, however, significant only in the second experiment (Table 1). EHDP had no significant effect on the chromatographic distribution of radioactivity in plasma, liver and kidney.

**Discussion**

The stimulatory effect of a low dose of EHDP [16 $\mu$mol ($\approx$ 1 mg of P)/kg s.c. daily for 7 days] on calcium absorption observed in duodenal segments...
Calcium absorption in rats

TABLE I. Distribution of intestinal metabolites 24 h after injection of 25-[3H]hydroxycholecalciferol in rats pretreated with ethane-1-hydroxy-1,1-diphosphonate

Ethane-1-hydroxy-1,1-diphosphonate (EHDP) was given s.c. daily for 7 days [16 μmol (= 1 mg of P)/kg body wt.]. Results (mean value ± SEM) represent the percentage of radioactivity recovered in the fraction of the different peaks from the Sephadex LH-20 column. Significance of results: *P < 0.05, **P < 0.02, ***P < 0.001, as compared to the corresponding control peak value. n.d., not detected; 25-(OH)D₃, 25-hydroxycholecalciferol; 24,25-(OH)₂D₃ and 1,25-(OH)₂D₃, 24,25- and 1,25-dihydroxycholecalciferol.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 4</td>
<td>n.d.</td>
<td>73·3±3·5</td>
<td>13·9±2·0</td>
<td>6·6±0·4</td>
<td>6·9±1·4</td>
<td></td>
</tr>
<tr>
<td>EHDP 4</td>
<td>n.d.</td>
<td>69·2±3·9</td>
<td>11·3±1·4</td>
<td>12·0±1·8 *</td>
<td>8·4±1·4</td>
<td></td>
</tr>
<tr>
<td>Control 6</td>
<td>4·4±0·3</td>
<td>65·9±0·9</td>
<td>13·6±0·6</td>
<td>10±2±0·9</td>
<td>6·0±0·4</td>
<td></td>
</tr>
<tr>
<td>EHDP 6</td>
<td>3·9±0·3</td>
<td>61·6±1·2 **</td>
<td>12·1±0·4</td>
<td>15·4±0·5 ***</td>
<td>6·0±0·3</td>
<td></td>
</tr>
</tbody>
</table>

(1) Each extract is made of pooled intestine of two rats.

with the tied-loop in situ technique confirms results obtained with a balance study (Gasser et al., 1972). The same low dose also increases, in the intestine, the amount of a vitamin D₃ metabolite eluted from Sephadex LH-20 columns in the position of 1,25-(OH)₂D₃. As 25,26-dihydroxycholecalciferol, the other dihydroxy metabolite known to be eluted from Sephadex LH-20 at the same position as 1,25-(OH)₂D₃, has not been detected in the intestine (Haussler & Rasmussen, 1972) the observed difference should correspond to increased 1,25-(OH)₂D₃. Thus the increased rate of calcium transfer in the gut might well be mediated through a rise in production or an inhibition of the metabolism of 1,25-(OH)₂D₃.

This observation may be of importance with respect to the clinical application of EHDP for treatment of some disorders of calcium metabolism. Indeed, in patients with Paget’s disease EHDP given at a daily dose of 80 μmol (20 mg) of compound/kg orally, a dose corresponding approximately to 0·15 mg of P/kg given daily s.c. has also a stimulatory effect on calcium absorption (Szymendera, Heaney & Saville, 1972; Guncaga, Lauffenburger, Lentner, Dambacher, Haas, Fleisch & Olah, 1974).

The reason for the dose-dependent opposite effect of EHDP on the intestinal accumulation of 1,25-(OH)₂D₃ and calcium absorption is still unknown. It is interesting that large doses of EHDP which prevent the retention of calcium in the skeleton (Gasser et al., 1972) inhibit the production of 1,25-(OH)₂D₃, whereas smaller doses which enhance calcium retention in bone could tend to augment the formation of the vitamin D₃ metabolite most active in stimulating calcium absorption. Whether the effects of EHDP on the synthesis and maybe the metabolism of 1,25-(OH)₂D₃ are secondary to the bone action of the diphosphonate remains to be elucidated.

Acknowledgments

The authors thank particularly Mr D. Mously, Mrs I. Tschudi and Miss I. Pagani for their expert technical assistance, and Mrs B. Gyger for preparing the manuscript. This work has been supported by the U.S. National Institutes of Health (grant no. AM 07266), the Schweizerischer Nationalfonds für wissenschaftliche Forschung (grant no. 3.326.70), and by the Procter & Gamble Co.

References


