1,25-DIHYDROXYCHOLECALCIFEROL AND INDUCTION OF ALKALINE PHOSPHATASE IN ORGAN CULTURE

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It is well known that intestinal alkaline phosphatase is induced during development (1). In birds, its activity begins to rise shortly before hatching and then increases dramatically at the time of hatching (2, 3). Recently, we showed that an active metabolite of vitamin D₃ is required for this process (3). The present report describes further investigations which were designed to determine the quantitative relationship between vitamin D₃ active metabolites and alkaline phosphatase activity using an organ culture system of embryonic chick duodenum.

Chick embryos and the organ culture procedure have been described in detail elsewhere (3). Briefly, duodena from 20-day chick embryos were slit open longitudinally and placed with the mucosal side up on a stainless steel grid in a petri dish. Each one-half of duodenum was cultured in medium (Eagle’s MEM) with or without the sterols. Vitamin D₃, 25-OH-D₃ and 1,25-(OH)₂D₃ were dissolved in ethanol and added to the culture media in the concentration shown in Fig. 1. The duodena on the grids were cultured for 48 hr in a humidified incubator continuously gassed with 5% CO₂ in air. Alkaline phosphatase activity and protein concentration of duodenal homogenate were determined as described in the previous report (3). The enzyme activity was expressed as the ratio of treated and control duodena (relative activity).

1,25-(OH)₂D₃ produced a graded increase in the duodenal alkaline phosphatase in culture at concentrations of 0.625 to 62.5 nm (Fig. 1). In contrast to 1,25-(OH)₂D₃, 25-OH-D₃ and D₃ did not give any significant response at the effective level of 1,25-(OH)₂D₃, although a small response to 25-OH-D₃ was observed at 625 nm. Relative activity of alkaline phosphatase induced by 25-OH-D₃ and D₃ at 625 nm was 1.21 and 1.06, respectively. Those values were extremely lower than that of 1,25-(OH)₂D₃ (1.90). On the other hand, in the previous experiment, 1α-OH-D₃ (synthetic active metabolite of D₃) was used instead of 1,25-(OH)₂D₃ and a comparable response was observed at the concentration of 62.5 nm (3).

Abbreviations used: D₃, vitamin D₃; 25-OH-D₃, 25-hydroxycholecalciferol; 1,25-(OH)₂D₃, 1,25-dihydroxycholecalciferol; 1α-OH-D₃, 1α-hydroxycholecalciferol; CaBP, calcium binding protein.
Fig. 1. Induction of alkaline phosphatase by D₃, 25-OH-D₃ and 1,25-(OH)₂D₃ during organ culture. Duodena from 20-day chick embryos were cultured for 48 hr with or without the sterols at the indicated concentrations. Each point indicates the mean and vertical line of one standard error for the ratio of treated to control culture. Data are pooled from 6 culture experiments. ■—■, D₃; △—△, 25-OH-D₃; ○—○, 1,25-(OH)₂D₃; ×, 1,25-(OH)₂D₃ with D₃.

These results suggest that 1-hydroxylation on the molecule of D₃ is essential for the induction of intestinal alkaline phosphatase during chick development at a physiological dose level. 1α-OH-D₃ was probably effective because of the ability of chick intestine to hydroxylate at C-25 of D₃ in vitro (4).

Furthermore, in order to evaluate whether the linear relationship which was shown in that extent will be able to reproduce in the presence of other sterols in the same medium, the effect of 1,25-(OH)₂D₃ was examined at 62.5 nM in the presence of D₃ at the concentration of 650 nM, ten times excess of 1,25-(OH)₂D₃. As shown in the figure, significant D₃ interference was not observed in the alkaline phosphatase response to 1,25-(OH)₂D₃. Consequently, the linear relationship between the dose of 1,25-(OH)₂D₃ and alkaline phosphatase activity could be kept in the presence of other ineffective sterol, such as D₃ or 25-OH-D₃.

Thus, this result suggests that alkaline phosphatase can be used as quantitative index of 1,25-(OH)₂D₃ in serum and other biological specimens. Induction of CaBP in duodenal culture of chick embryos (5) and stimulation of bone resorption in fetal rat bone culture (6) may be included as other biological quantitative indices of 1,25-(OH)₂D₃. However, the advantage of alkaline phosphatase for the quantitation of 1,25-(OH)₂D₃ would be the simplicity of its assay procedure.

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