RELATIONSHIP OF INTESTINAL AND PLASMA CALCIUM-BINDING PROTEIN TO INTESTINAL CALCIUM ABSORPTION

A. BAR, A. MAOZ and S. HURWITZ

Institute of Animal Science, Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel

Received 9 March 1979

1. Introduction

Vitamin D-dependent calcium-binding proteins (CaBP) have been identified in the intestines of birds [1–4] and mammals [2,5,6]. The same or similar CaBPs have been found in other organs with a high ability to transport calcium, such as kidney [7–9], shell gland [10–12], placenta [13] and mammary glands [14]. In addition, CaBP has been found in chick brain [15], blood plasma and bone [16].

In vitamin D-deficient chicks the appearance of intestinal CaBP following a single injection of some cholecalciferol metabolites, is not parallel to the changes in calcium absorption [17,18]. However, in vitamin D-fed animals, intestinal CaBP concentration parallels, in most cases, the calcium absorption capacity [2,19,20] measured either by the loop technique in situ [19] or by using non-absorbed reference substances in vivo [3,20].

Here, an assessment was made of the relationship of duodenal CaBP of cholecalciferol-fed chicks maintained on various dietary calcium intakes, to calcium absorption capacity or to the actual amount of calcium absorbed in vivo. In addition, the possible relationship between duodenal and blood plasma CaBP was evaluated.

2. Experimental

Day 1 male chicks were fed for 7 days a diet containing 40 μg/kg of cholecalciferol diet, 1.1% calcium and 0.7% phosphorus. On day 8 the birds were divided into 5 lots which were fed for an additional 8 days diets containing 40 μg/kg cholecalciferol, 0.7% phosphorus, 25 μCi/kg ^{144}Ce as a non-absorbed reference substance, and 0.33, 0.69, 0.86, 1.39 and 2.07% calcium, respectively.

At day 16 heparinized blood samples were obtained from 7 birds of each group which were then killed by an overdose of sodium pentobarbital. The contents of the lower ileum were analyzed for calcium and ^{144}Ce and the net calcium absorption was calculated [3]. Calcium in plasma and intestinal content was determined by EGTA titration using an automatic titrator (Precision Systems, USA). The duodenal mucosa was homogenized and assayed for CaBP by a radioimmunoassay [20]. This assay, sensitive to as low as 30 pg CaBP, was also used to measure the concentration of CaBP in the blood plasma using a lower antiserum concentration (final dilution of 1/4500 and 1/90 000 for mucosa and plasma, respectively).

3. Results and discussion

Plasma calcium (fig.1) increased as dietary calcium intake increased, to reach a plateau starting from an intake of ~600 mg/chick. Plasma CaBP concentration of the cholecalciferol-fed chicks was within 15–56 ng/ml* and, similarly to duodenal CaBP, decreased as dietary calcium increased (fig.1). The total daily amount of absorbed Ca increased with intake up to 800 mg/day, while % calcium absorption decreased.

Thus, duodenal CaBP and plasma CaBP were

* Duodenal and plasma CaBP concentrations of cholecalciferol-deficient chicks were 21 ± 4 μg/g and 3.3 ± 0.3 ng/ml, respectively

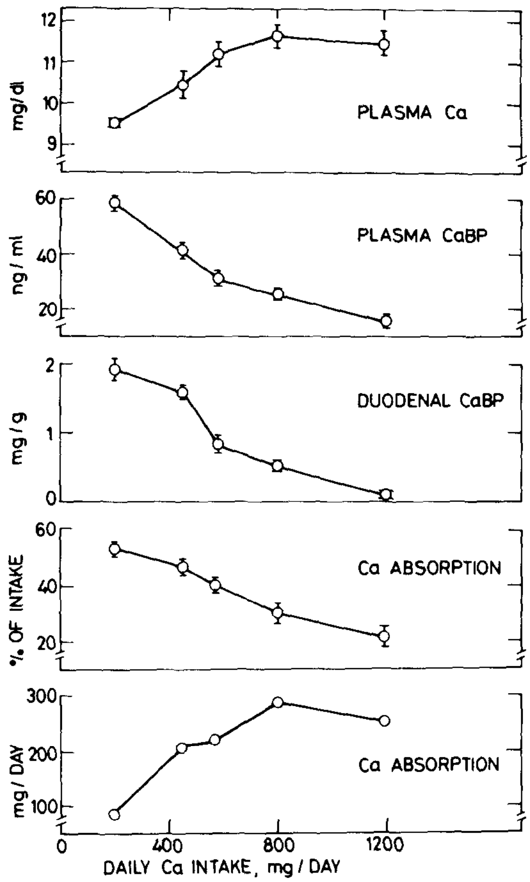

Fig 1 Plasma calcium, intestinal and plasma CaBP and intestinal calcium absorption as functions of calcium intake in chicks The mg calcium absorbed/day was calculated from the individual values of % absorption multiplied by the average daily intake of calcium and therefore the SE values are not given

positively correlated to % of net calcium absorption and negatively correlated to the daily amount of calcium absorbed (fig 1) These relationships do not mean necessarily that CaBP is involved directly in the calcium absorption process. Nevertheless, the concentration of intestinal CaBP in vitamin-fed chicks is associated with the increased capacity of the intestine to absorb calcium (fig.1) [19,20], rather than with the actual amount of calcium absorbed, as suggested [17]

A highly significant ($P < 0\ 01$) correlation was obtained between duodenal and plasma CaBP, on the

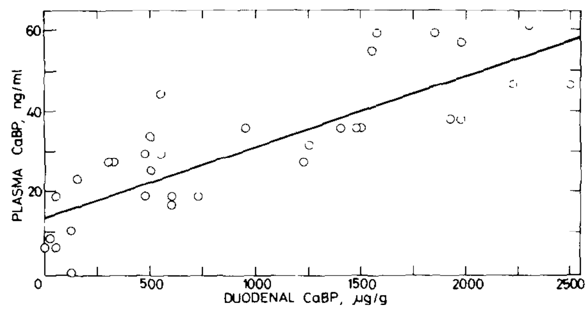

Fig 2 The relationship between duodenal and plasma CaBP The regression equation is

Plasma CaBP (ng/ml) = 13 655 ± 0 0192 × duodenal (µg/g) CaBP

Correlation coefficient = 0 825, t value = 8 401, DF = 33

basis of values obtained from individual chicks (fig 2) This suggests that measurements of plasma CaBP can replace, under certain conditions, measurements of intestinal CaBP, as a non-destructive procedure

Acknowledgements

Contribution no 240-E, 1978 series, from the Agricultural Research Organization, The Volcani Center, Bet Dagan This research was supported by the National Poultry Marketing Board The specific antiserum was generously donated by Professor R. H Wasserman of Cornell University, Ithaca, NY We are grateful to Mrs Marcella Cotter and Mrs Ruhama Drylich (Yemini) for their skilled technical assistance

References

[1] Wasserman, R H and Taylor, A N (1966) Science 152, 791–793
[2] Wasserman, R H, Taylor, A N and Fullmer, C S (1974) Biochem Soc Spec Publ 3, 55–74
[3] Bar, A, Dubrov, D, Eisner, U and Hurwitz, S (1976) Poultry Sci 55, 633–638
[4] Bar, A, Dubrov, D, Eisner, U and Hurwitz, S (1978) J Nutr 108, 1501–1507
[5] Hitchman, A J W and Harrison, J E (1972) Can J Biochem 50, 758–765
[6] Fullmer, C S and Wasserman, R H (1975) Biochim Biophys Acta 393, 134–142

[7] Taylor, A. N. and Wasserman, R. H. (1972) Am. J. Physiol. 223, 110–114.
[8] Piazolo, P., Schleyer, M. and Franz, H. E. (1971) Hoppe-Seyler's Z. Physiol. Chem. 352, 1480–1486.
[9] Hersdorf, C. L. and Bronner, F. (1975) Biochim. Biophys. Acta 379, 553–561.
[10] Corradino, R. A., Wasserman, R. H., Pubols, M. H. and Change, S. I. (1968) Arch, Biochem. Biophys. 125, 378–380.
[11] Bar, A. and Hurwitz, S. (1973) Comp. Biochem. Physiol. 45A, 579–586.
[12] Fullmer, C. S., Brindak, M. E., Bar, A. and Wasserman, R. H. (1976) Proc. Soc. Exp. Biol. Med. 152, 237–241.
[13] Bruns, M. E. H., Furora, A. and Avioli, L. V. (1978) J. Biol. Chem. 253, 3186–3190.
[14] Bauman, V. K., Valinience, M. Y. and Pastvhov, M. V. (1972) Proc. Latvian Acad. Sci. 294, 133–134.
[15] Taylor, A. N. (1974) Arch. Biochem. Biophys. 161, 100–108.
[16] Christakos, S. and Norman, A. H. (1978) Science 202, 70–71.
[17] Spencer, R., Charman, M., Wilson, P. W., Lawson, D. E. M. (1978) Biochem. J. 170, 93–101.
[18] Morrissey, R. L., Zolock, D. T., Bikle, D. D., Empson, R. L. and Bucci, T. J. (1978) Biochim. Biophys. Acta 533, 23–33.
[19] Morrissey, R. L. and Wasserman, R. H. (1971) Am. J. Physiol. 220, 1509–1515.
[20] Bar, A. and Hurwitz, S. (1979) Endocrinology, in press.