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Vitamin-D-Dependent Calcium-Binding-Protein and Parvalbumin Occur in Bones and Teeth

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SUMMARY

Vit-D-Dependent calcium binding protein and parvalbumin have both been detected in ameloblasts and calcified cartilage by immunohistochemical techniques. These two Ca^{2+} binding proteins may play a crucial role in the local accumulation of Ca^{+2} ions during the process of mineralization.

The mechanisms underlying the deposition of inorganic substances in bone and teeth during physiologic calcification are still the object of intense debate [1,2]. The hypotheses concerning the factors controlling the initiation of mineralization can be subdivided into three large categories: enzymatic (or non-enzymatic) local elevation of phosphate and calcium [3,4,5,6,7], enzymatic removal of inhibitors of calcification [8] and direct nucleation of $CaPO_4$ crystals on collagen fibrils [9].

In support of the first line of thought we report here the simultaneous occurrence of two different very high affinity Ca^{2+} binding proteins [vitamin-D-dependent CaBP=VD CaBP and parvalbumin=PV] in bones and teeth. During the studied age period and with immunohistochemical methods, we detected the proteins only in calcified cartilage of bones and in ameloblasts of teeth. We propose that VDCaBP and PV help increase the Ca^{2+} concentration at the calcification front in some regions involved in mineral deposition.

MATERIAL AND METHODS

Whole rat embryo's, fetuses, newborns and tissue of young and adult rats of both sexes of the Wistar strain (Ivanovas GMBH, Kisslegg, BRD) were used for this study. They were fixed by immersion in (or perfusion with) Bouin's fluid. After dehydratation the largest specimens were cut in the mid-sagittal plane before embedding in paraffin, whereas the smaller were embedded in toto. All postnatal (and some of the prenatal) specimens were decalcified with 10% formic acid for varying periods of time prior to embedding in paraffin. Sections were mounted on slides and incubated with probed solutions of the antisera (1:2,000 to 1:20,000) and further processed according to the unlabeled antibody method as extensively described in [10]. The parvalbumin and the Vit-D-Dep. CaBP (28,000 MW) antisera have been characterized and used in various publications [10,11,12,13] and do not crossreact in an immunoblotting of bone and tooth extracts. Immunohistochemical controls comprised the use of preimmune sera of the same animals which gave the antiserum and adsorbed antisera as described [10,11].

RESULTS

With these immunohistochemical methods we found PV and VDCaBP immunoreactivities limited exclusively to the ameloblasts in teeth (Fig. 1) and to the calcified cartilage in bone (Fig. 2). The staining with both antisera was identical in both locations. All adamantoblasts are equally well stained and the immunoreaction is characteristically polarized towards the apex of the cells. This region harbours the largest quantity of PV and VDCaBP as judged from antisera dilutions experiments. Even the cell nuclei (but not the nucleoli) display VDCaBP (Fig. 1) and PV immunoreactivities. The preenamel matrix and enamel of decalcified teeth do not show any staining with both antisera. Undecalcified teeth could not be studied with the present method. In undecalcified bones, PV (Fig. 2) and VDCaBP immunoreactivities occur extracellularly in round structure of 3-5 µm diameter (calcospherites) and in fused aggregates of them, which form the calcified trabeculae. Single chondroclasts and probably also some preosteoblast displayed moderate immunoreactivity. In decalcified bone no immunostaining could be detected. A thorough study of undecalcified postnatal bone was not possible, because of technical constraints.

DISCUSSION

This preliminary communication demonstrates by immunohistochemical methods the occurrence of two different Ca^{2+} binding proteins in calcifying tissues. Biochemical support for this morphological study has been derived from two-dimensional gel electrophoresis and high-pressure liquid chromatography of tissue extracts (Heizmann et al, in preparation) and (for VDCaBP) also by radioimmunoassay [14]. The

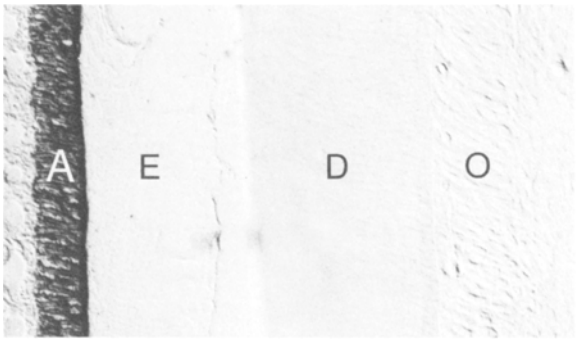

Fig. 1. Longitudinal paraffin section of the lower incisor of the decalcified rat head (+21 days). The resorptive ameloblasts (A) are intensely labeled by VDCaBP antiserum, particularly in their apical portion. Note the staining of the ameloblast nuclei and the unstained, reticular organic remnants of the decalcified enamel (E). Odontoblasts (O) and dentin (D) lack immunoreactivity. The staining pattern with PV antisera was identical.

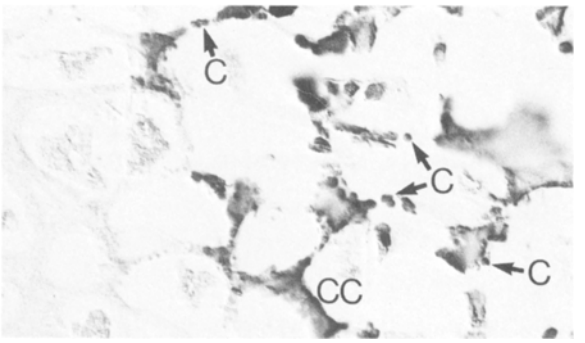

Fig. 2. Longitudinal section through the body of a thoracal vertebra of an undecalcified rat fetus (-1 day). Single, round globules (calcospherites, C) and strands of calcified cartilage (CC) are homogeneously PV immunoreactive. Identical immunoreaction was found after incubation with VCDaBP antisera.

immunohistological procedure applied in this study fully preserved antigenicity as has been shown previously [10,11] and decalcification did not destroy the intracellular antigen as tested in identically processed control brain and muscle tissues (not shown). However, it is not as yet clear to what degree the decalcification fluid "extracted" the extracellularly located Ca^{2+}-binding proteins along with mineral salts. Therefore our inability to demonstrate PV and VDCaBP at other sites i.e. calcified osteoid, may reflect merely an inadequacy of the actual technique. PV and VDCaBP are structurally homologous to calmodulin; all three proteins occur in various tissues [10,11,12,13] but their physiological role is at present unclear. Parvalbumin in muscle is possibly involved in fast relaxation [11] and VDCaBP is likely to be concerned with the transfer of Ca^{2+} across epithelial membranes [13]. However, the widespread occurrence of both proteins in many different locations indicates also a role in other kinds of cellular events involving calcium. Their overlapping localization in bones and teeth may suggest integrated functions, which could affect the binding, transport, accumulation, delivery and eventual precipitaton of Ca^{2+} at the calcification front.

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