SPECIFIC BINDING-PROTEIN FOR CHOLECALCIFEROL IN MAMMARY GLANDS OF LACTATING RATS

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1. Introduction

Recent findings have shown that vitamin D (cholecalciferol) is a prohormone which gives rise to $25(OH)D_3$* and to $1,25(OH)_2D_3$. The dihydroxylated metabolite which is produced by the kidney [1] is the active hormonal form of the vitamin which carries out all the known biological functions of the vitamin.

Since the above findings were reported, several studies were carried out in order to establish whether specific binding proteins for these fatsoluble steroids are present in tissues, similar to what is known with regard to other steroid hormones. It has been possible to show that blood and cytosol fractions of other tissues do contain such proteins [2–4], but, apart from chick blood, which was found to contain a specific binding protein for cholecalciferol [5,6], all the proteins tested were found to possess high binding affinity towards $25(OH)D_3$ [3]. As no specific hormonal function can be related to $25(OH)D_3$, the biological significance of these binding proteins remains unknown.

Previous studies from this laboratory [7] have shown that mammary glands of lactating rats contain mainly cholecalciferol, and the present study reports on the presence of a specific binding protein(s) for this steroid in these glands. The biological significance of the protein in the lactating state is discussed.

* *Abbreviations:* $25(OH)D_3$ – 25-hydroxycholecalciferol; $1,25(OH)_2D_3$ – 1,25-dihydroxycholecalciferol; $24,25(OH)_2D_3$ – 24,25-dihydroxycholecalciferol.

2. Materials and methods

2.1. *Animals and preparation of mammary glands cytosol*

Lactating rats (Wistar), fed Purina rat chow, were used for the preparation of mammary glands cytosol. After killing, the glands were dissected out, rinsed with saline, weighed, minced and homogenized in 0.02 M sodium phosphate buffer pH 7.6. The homogenate was then centrifuged at 105 000 *g* for 1.5 h in an SW 36 rotor in a Beckman centrifuge, and the supernatant obtained was used for the gel-electrophoresis analysis and for the competitive displacement studies.

2.2. *Gel-electrophoresis and competitive displacement studies*

The cytosol preparation was mixed with [1,2-^{3}H] cholecalciferol (The Radiochemical Centre, Amersham, U.K., sp. act. 12.6 Ci/mmol) and kept at 4°C for 16 h. The cytosol was then subjected to polyacrylamide-disc-gel electrophoresis in a continuous buffer system [8] and, at the completion of the run, the gels were frozen with solid CO_2 and 1 mm segments were sliced and counted for radioactivity [6]. In order to assess the binding affinity of the protein(s), competitive displacement studies of protein bound [26,27-^{3}H] $25(OH)D_3$ (The Radiochemical Centre, Amersham, U.K., sp. act. 12.3 Ci/mmol) were carried out using charcoal coated with dextran as previously described [9].

3. Results

The existence of a specific binding protein(s) for cholecalciferol in cytosol prepared from mammary glands of lactating rats was readily demonstrated by polyacrylamide-disc-gel electrophoresis following incubation of the cytosol with radiolabelled cholecalciferol (fig.1). The protein(s) which binds the steroid possessed α-globulin mobility, and the high binding affinity towards cholecalciferol could already be seen from the distribution of the radioactivity along the gel. Despite the low solublity of cholecalciferol in water, the radioactivity recovered was found to be distributed between the protein(s) possessing α-globulin mobility and lipoproteins which remain at the origin. When the incubated mammary gland cytosol was mixed with rat serum, or rat skin cytosol previously incubated with [4-^{14}C] cholecalciferol, and run on the gels, the ^{14}C radioactivity was found to be distributed all along the gels, with very little in association with the ^{3}H peak.

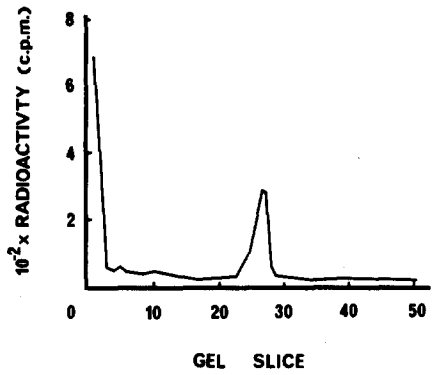

Fig.1. Distribution of tritium on the polyacrylamide-disc-gel electrophoresis of mammary glands cytosol incubated in vitro with [1,2-^{3}H]cholecalciferol. For experimental details, see section 2.2.

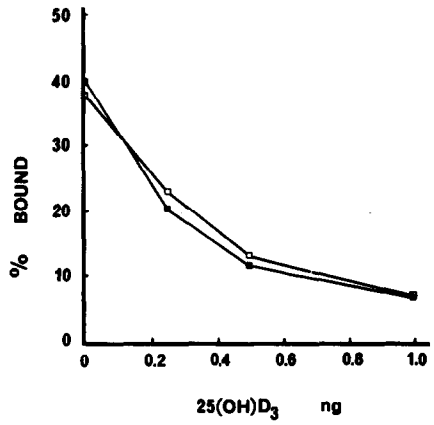

Fig.2. Competitive displacement of [26,27-^{3}H]25(OH)D$_3$ from rat serum and mammary glands binding proteins. For experimental details, see section 2.2. (■——■) Mammary glands; (□——□) Serum.

Table 1
Association constants of serum and mammary glands binding proteins towards 25(OH)D$_3$

Source of binding-proteins	$K_{assoc.}$ (M^{-1})
Serum	2.2×10^{9}
Mammary glands	3.5×10^{9}

That high affinity binding is involved was subsequently demonstrated by analysis of competitive displacement curves [10] (fig.2). Due to the virtual insolubility of cholecalciferol in water, whereas 25(OH)D$_3$ with its additional polar group has sufficient solubility to allow binding to occur, the competitive displacement studies were carried out with 25(OH)D$_3$. As can be seen from table 1, the association constant of this steroid with mammary gland cytosol is higher than the one obtained with rat serum.

4. Discussion

It seems clear from the present study that mammary glands of lactating rats contain a specific binding protein(s) for cholecalciferol. Despite the low solubility of this steroid in water, 16 h in vitro incubation of the steroid with the cytosol preparation were sufficient to bind most of the molecules to the protein(s), excluding some that were trapped on the lipoproteins. When similar experiments were carried out with other known vitamin D binding proteins, including the specific cholecalciferol-binding-protein from chick serum, the molecules were found to be bound to many other nonspecific proteins as well [2,3,6].

This finding that mammary glands of lactating rats

contain a specific binding protein(s) for cholecalciferol, possessing higher binding affinity than the serum binding protein, explains previous observations that 90% of the total metabolites of vitamin D found in mammary glands of lactating rats is in the form of the unchanged vitamin, while at the same time the blood contains mainly $25(OH)D_3$ and $24,25(OH)_2D_3$ with very little cholecalciferol [7].

It is suggested, therefore, that the biological role of this protein(s) in the lactating state is to pick up this steroid selectively from the circulation in order to ensure its supply, via the milk, to the newborn suckling pups, who can then metabolise the molecule to its hydroxylated forms, as was previously shown [7].

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