J. steroid Biochem. Vol. 27, No. 4–6, pp. 807–813, 1987
Printed in Great Britain.
0022-4731/87 $3.00 + 0.00

Proceedings of the VII International Congress on Hormonal Steroids (Madrid, Spain, 1986)

23. The Action of and Resistance to Vitamin D

DEVELOPMENTAL CHANGES IN RESPONSIVENESS TO VITAMIN D METABOLITES

DALIA SÖMJEN, YEHUSHUA EARON*, SHAUL HARELL*, ZVI SHIMSHONI, YOSEF WEISMAN†, ARIE HARELL, ALVIN M. KAYE‡ and ITZHAK BINDERMAN

*Hard Tissues Unit, Department of Pediatrics, Ichilov Hospital, 6 Weizmann Street, Tel Aviv 64239, Israel, and †Vitamin Research Laboratory, Ichilov Hospital, Tel Aviv 64239, Israel, and ‡Department of Hormone Research, The Weizmann Institute of Science, Rehovot 76100, Israel

Summary—We have demonstrated that epiphyseal chondroblasts contain specific receptors for 24R,25-dihydroxy vitamin D_3(24,25$(OH)_2D_3$) while diaphyseal osteoblasts contain specific receptors for 1α25-dihydroxy vitamin D_3 (1,25$(OH)_2D_3$). Both metabolites induce DNA synthesis and creatine kinase (CKBB) activity. We have also found that the responsiveness of rat kidney to these metabolites changes during development. In embryonic and early postnatal stages, the kidney responds to 24,25$(OH)_2D_3$, later to both 24,25$(OH)_2D_3$ and 1,25$(OH)_2D_3$, and the mature kidney only to 1,25$(OH)_2D_3$. These responses correlate with changes in the specific receptors present in the kidney.

Furthermore, we have compared developmental changes in skeletal (epiphysis, diaphysis and mandibular condyle) and non-skeletal (kidney, cerebellum, cerebrum, liver and pituitary) tissue in both rat (a postnatal developer) and rabbit (a perinatal developer). Epiphyseal or diaphyseal chondroblasts at any stage of development were predominantly responsive to 24,25$(OH)_2D_3$, whereas osteoblasts were responsive to 1,25$(OH)_2D_3$. In contrast, condylar chondroblasts, kidney, cerebellum and pituitary responded to 24,25$(OH)_2D_3$ during early development and subsequently developed responsiveness to 1,25$(OH)_2D_3$.

Using primary cell cultures from kidneys at different stages of maturation, we showed the same developmental pattern as *in vivo*. Chronic treatment of the cells with 24,25$(OH)_2D_3$, but not 1,25$(OH)_2D_3$, caused precocious development of responsiveness to 1,25$(OH)_2D_3$ in culture. We suggest that 24,25$(OH)_2D_3$ acts as a maturation factor, during early development in kidney, and probably in other tissues, possibly by induction of receptor to 1,25$(OH)_2D_3$, accompanied by down-regulation of its own receptor.

INTRODUCTION

Vitamin D is a prohormone for the more polar metabolites, 1α,25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$) and 24R,25-dihydroxyvitamin D_3 (24,25$(OH)_2D_3$), the principal mediators of the biological activities originally ascribed to the parent compound. There is substantial evidence that the mechanism of action of both metabolites is similar to that of other steroid hormones, initiated by interaction with specific intracellular receptors. The tight association of a steroid receptor complex with specific chromatin loci in the nuclei of responsive cells leads to the modulation of mRNA synthesis for specific proteins which have been shown to be involved in the biological responses of vitamin D metabolites [1]. 1,25$(OH)_2D_3$ has a major role in calcium homeostasis through its effects on calcium absorption in the intestine [2–8] and on bone mineral mobilization [9, 10]. However, many more organs were found to have receptors for 1,25$(OH)_2D_3$ [1, 11] leading to the realization that the vitamin D endocrine system extends far beyond its original classical sites of action [1]. Furthermore, recent studies indicate that vitamin D metabolites are associated with cell proliferation and differentiation [1].

The more recently recognized vitamin D metabolite, 24,25$(OH)_2D_3$, which is not implicated in calcium transport, was shown to have important roles in development of endochrondral bone [12–14] and hatching of chick embryos [15, 16]. Specific receptors for this metabolite were identified in parathyroid gland [17] chondrocytes [18], epiphyseal growth plates [19] and limb bud mesenchymal cells [20]. It was therefore intriguing to test for responsiveness to both vitamin D metabolites during postnatal development of the rat kidney and skeletal tissues. It was of particular interest to compare the responsiveness of the brain in the rat, which is a postnatal developer, with brain of the rabbit which, like the human, is a perinatal developer, with a brain growth spurt from 10 days before birth to 30 days after birth [21]. In order to define the developmental role of 24,25$(OH)_2D_3$, we have also investigated its influence on the development of responsiveness to 1,25$(OH)_2D_3$ in kidney cell cultures.

RAT BONE TISSUES

We have previously shown that 1,25$(OH)_2D_3$ shows specific binding [19] and induces DNA synthesis, and ODC [22] and creatine kinase (CKBB) activity [23], in diaphyseal osteoblasts of vitamin D-depleted rats, at all ages. On the other hand, 24,25$(OH)_2D_3$ is active by the same criteria [22, 24] in chondroblasts from epiphyseal cartilage or chick mesenchymal cells [20, 25, 26] at all ages measured. Rat epiphyseal or diaphyseal chondroblasts at any

stage of development were predominantly responsive to $24,25(OH)_2D_3$, whereas osteoblasts were responsive to $1,25(OH)_2D_3$ [19, 22]. In contrast, condylar chondroblasts (see below) show a developmental change in responsiveness, responding to $24,25(OH)_2D_3$ in early development and subsequently developing responsiveness to $1,25(OH)_2D_3$. Therefore, it seems that epiphyseal chondroblasts respond similarly to embryonic and "young" tissues while bone-derived osteoblasts respond similarly to "adult" mature tissues.

RAT KIDNEY

The kidney is the principal site of synthesis of the dihydroxylated metabolites of vitamin D [27–29]. In addition, adult kidney contains receptors for $1,25(OH)_2D_3$ [30–34], detectable both in cytoplasmic and nuclear preparations. An autocrine response to $1,25(OH)_2D_3$ is shown by mouse kidney by increased synthesis of two vitamin D dependent calcium binding proteins [35]. The kidney of the rat also contains a vitamin D dependent calcium binding protein [36–38] similar to those found in the intestinal mucosa [39]. The concentration of renal calcium binding protein [35], similarly to intestinal calcium binding protein [40], changes with postnatal development, showing a marked increase starting from day 18.

In kidney from vitamin D-depleted rats [41], there is a change in binding activity with age. $24,25(OH)_2D_3$ binds predominantly in renal cytosol of 3- to 15-day old rats with maximal binding at 12 days and shows minimal specific binding from 21 to 35 days. By contrast $1,25(OH)_2D_3$ is bound in increasing concentrations with age till a plateau is reached at 18 days. At 18 days, there is also a cross-over point at which there is equal binding for the two metabolites [41]. Between 21 and 35 days, renal cytosol binds predominantly $1,25(OH)_2D_3$. These changes in binding during post-natal development are reflected in changes in responsiveness, using either stimulation of CKBB activity or of DNA synthesis as criteria.

Stimulation of CKBB activity

Both vitamin D-depleted and normally fed rats injected with either $1,25(OH)_2D_3$ or $24,25(OH)_2D_3$ show an age-dependent response (Fig. 1).

In normally fed rats (D+), the pattern of stimulation is similar to that found in vitamin D-depleted rats; first responsiveness to $24,25(OH)_2D_3$, followed by a switch to responsiveness to $1,25(OH)_2D_3$. In 1-day old vitamin-D depleted rats (D−), injected with either metabolite, renal CKBB is slightly decreased. At 2 days, no response to either metabolite is seen. In 7-day-old rats, $24,25(OH)_2D_3$ caused an increase in CKBB activity; in 8- to 15-day-old rats this increase reached 120%. Up to the age of 15 days, $1,25(OH)_2D_3$ did not stimulate renal CKBB activity; from 21 to 50 days, it was increased only by $1,25(OH)_2D_3$ while $24,25(OH)_2D_3$ was no longer effective (Fig. 1). One-day-old normally fed (D+) rats, injected with either metabolite, show no significant change in CKBB activity. In 7–20-day-old rats, CKBB activity is increased only by

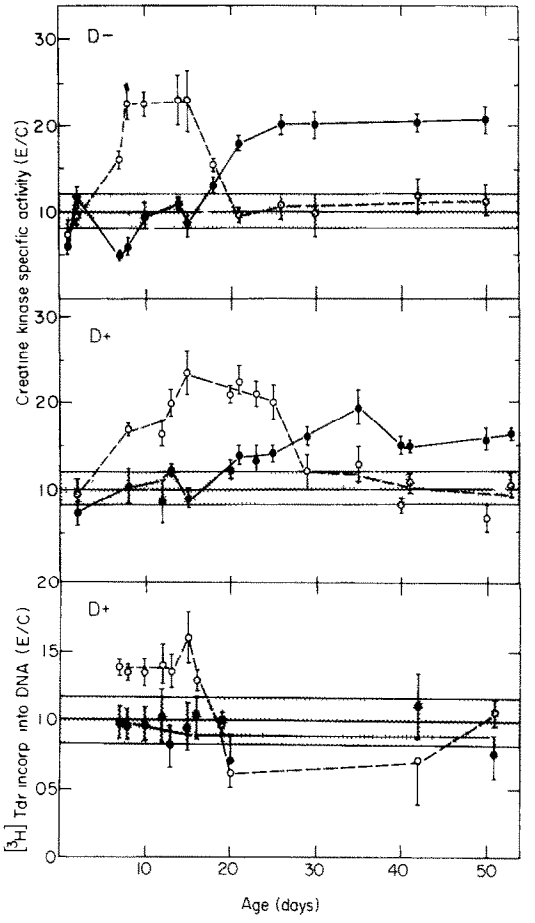

Fig. 1. Age-dependent stimulation by vitamin D metabolites of renal creatine kinase activity and DNA synthesis. Normally fed Wistar-derived rats (D+) were killed 24 h after injection of $1,25(OH)_2D_3$ (3 ng/g body wt), ●; or $24,25(OH)_2D_3$ (9 ng/g body wt), ○, or vehicle (20% ethanol in propylene glycol). These metabolites were kindly provided by Prof. S. Edelstein, Department of Biochemistry, The Weizmann Institute of Science. Vitamin D-depleted rats were raised as described previously [19]. All vitamin D-deficient animals (D−) had no detectable $25(OH)_2D_3$ (≤1.6 ng/ml) or $1,25(OH)_2D_3$ (≤5 pg/ml) in their serum; they were killed 24 h after injection of $1,25(OH)_2D_3$ (1 ng/g body wt [23]), ●; or $24,25(OH)_2D_3$ (3 ng/g body wt), ○. CKBB was extracted as described previously [41] and assayed at 30°C in a Gilford 250 automatic recording spectrometer at 340 nm using a coupled assay [23]. Unit enzyme activity was defined as the amount yielding 1 μmol ATP/min. In parallel experiments, DNA synthesis, measured as [^{3}H]thymidine (5 Ci/mmol; Radiochemical Centre, Amersham, Bucks, England) incorporation into acid-insoluble material, was assayed as described previously [25]. Results are expressed as experimental (E) divided by control (C, stippled bands) means ± SE for $n = 6$–9 (CKBB data from Sömjen *et al.* [41]).

$24,25(OH)_2D_3$ and not by $1,25(OH)_2D_3$. From 21 to 25 days after birth, renal CKBB is stimulated by both metabolites. However, in 29–52-day old rats, renal CKBB is stimulated exclusively by $1,25(OH)_2D_3$ (Fig. 1).

Stimulation of DNA synthesis

In 7–16-day-old rats [^{3}H]thymidine incorporation into renal DNA is increased by $24,25(OH)_2D_3$ but not by $1,25(OH)_2D_3$ (Fig. 1). Unlike the CKBB response (Fig. 1), from 19 days after birth there is no response to either metabolite by increased DNA synthesis (Fig. 1). This parallels the situation in uninephrectomy which causes renal hyperplasia only in young rats [42].

RAT CEREBELLUM

The sequential responsiveness found in the rat kidney led to analyses of the cerebellum, since calcium binding proteins [43, 44], similar to those induced by $1,25(OH)_2D_3$ in the intestine, were also reported in the brain. An increase in immunoreactive calcium binding protein was found [45] in chick cerebellum following chronic, but not acute, administration of vitamin D to severely vitamin D-deficient chicks. Rat brain nuclei, which contain receptors for $1,25(OH)_2D_3$ and/or calcium binding protein, showed an increase in choline acetyltransferase activity after 1 week of treatment with $1,25(OH)_2D_3$ [46].

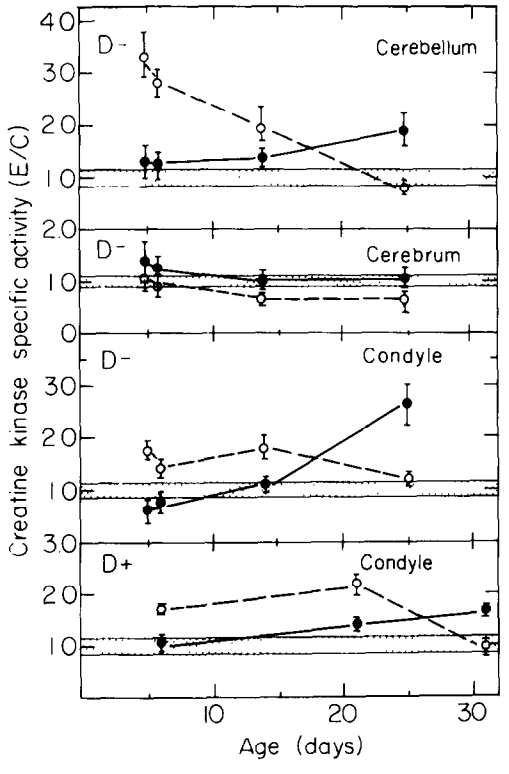

Fig. 2. Age-dependent stimulation by vitamin D metabolites of cerebral, cerebellar and condylar CKBB activity. Condylar CKBB activity was measured in normally fed (D+) and vitamin D-depleted (D−) rats. Experimental details are given in the legend to Fig. 1. ●, $1,25(OH)_2D_3$: ○, $24,25(OH)_2D_3$. Results are expressed as experimental (E) divided by control (C, stippled bands) means ± SE for $n = 4$–6 (Sömjen *et al.*, submitted for publication).

Vitamin D-depleted rats injected with $1,25(OH)_2D_3$ or $24,25(OH)_2D_3$ show an age-dependent cerebellar response (Fig. 2). In 5- to 14-day old rats, CKBB activity is increased in the cerebellum only by $24,25(OH)_2D_3$ and not by $1,25(OH)_2D_3$ (Fig. 2). At 25 days, there is no response to $24,25(OH)_2D_3$, but CKBB activity in the cerebellum is increased by $1,25(OH)_2D_3$.

In cerebrum there is no response to any of the metabolites at any age (cf. below, rabbit cerebellum).

In experiments using rat pituitary in organ culture, we could demonstrate an increase of CKBB activity in 12-day-old organs only by $24,25(OH)_2D_3$ (from 2.2 to 4.2 U/mg protein) and at 60 days exclusively by $1,25(OH)_2D_3$ (from 3.1 to 4.8 U/mg protein). Thus, in the nervous system, parallel sequential changes in responsiveness to vitamin D metabolites appear to occur in pituitary and cerebellum, similar to the developmental changes shown in kidney.

RAT CONDYLE

Condyles of vitamin D-depleted rats (D−) injected with either $1,25(OH)_2D_3$ or $24,25(OH)_2D_3$ also show an age-dependent response (Fig. 2). This tissue differs from the endochondrial bone forming tissue, the epiphysis, in that the chondrocytes undergo calcification directly without going through the stage of bone formation. In 5–9-day old rats, CKBB activity is increased only by $24,25(OH)_2D_3$ and not by $1,25(OH)_2D_3$ (Fig. 2). At 25 days after birth, condylar CKBB is stimulated only by $1,25(OH)_2D_3$ (Fig. 2).

In normally fed (D+) 6-day-old rats, only $24,25(OH)_2D_3$ stimulates condylar CKBB activity while in 31-day-old rats, condylar CKBB is stimulated only by $1,25(OH)_2D_3$. At 21 days after birth, condylar CKBB is stimulated more by $24,25(OH)_2D_3$ than by $1,25(OH)_2D_3$.

RABBIT ORGANS

Since the rabbit is a perinatal developer, compared with the rat, in which the development of some organs (particularly the brain) is shifted postnatally, it was of interest to compare the response of rats and rabbits to the vitamin D metabolites. The same pattern of response is found in rabbits as previously seen in rats but shifted to the perinatal rather than the postnatal period.

Normally fed rabbits injected with either $1,25(OH)_2D_3$ or $24,25(OH)_2D_3$ show an age-dependent response of CKBB in cerebellum and kidney (Fig. 3) but not in cerebrum or liver (not shown). In the cerebellum, $24,25(OH)_2D_3$, but not $1,25(OH)_2D_3$, significantly increases CKBB activity

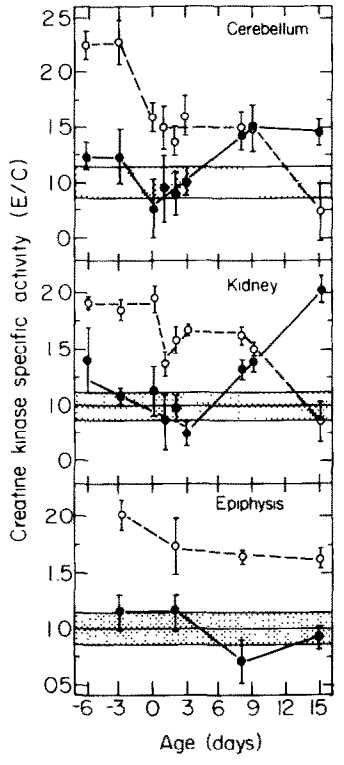

Fig. 3. Age-dependent stimulation by vitamin D metabolites of CKBB activity in cerebellum, kidney and epiphyses of normally fed rabbits. New Zealand white does were raised at the Tel Aviv University animal colony; fetuses or newborns from 6 days before to 15 days after birth were used. Rabbits were treated with the same doses as normally fed rats (see Fig. 1). To inject fetuses with the metabolites, a cesarean section was performed. Three groups of fetuses in each female were designated for i.p. injection of the metabolites or the vehicle into the abdomen of each fetus. The uterus was returned to the abdominal cavity and both muscle and skin closed with single stitches. Further experimental details are given in the legend to Fig. 1. Results are expressed as experimental (E) divided by control (C, stippled bands) means ± SE for $n = 6$–12, ●, $1,25(OH)_2D_3$; ○, $24,25(OH)_2D_3$. (Cerebellar data from Binderman *et al.*, submitted for publication; kidney and epiphysis data from Sömjen *et al.*, submitted for publication.

from −6d (6 days before birth) to 3 days after birth. At 15 days, only $1,25(OH)_2D_3$ increases CKBB activity, whereas at 8–9 days both metabolites stimulate the activity of CKBB (Fig. 3). The cerebrum (or liver) does not respond, at any age tested, to either of the metabolites.

The differences between the different brain regions in their response to vitamin D metabolites may be explained by differences in the pattern of development in the rabbit of the cerebrum and the cerebellum during the perinatal period up to 3 weeks of age [21]. During this time, the growth of the cerebellum is characterized predominantly by cell proliferation while in the cerebrum an increase in cell size is the main characteristic [21].

The increase in CK activity during the stage of most rapid cell division in the rabbit cerebellum (from 1.2 to 2.2 U/mg protein between −6 and 15 days) and its stimulation by $24,25(OH)_2D_3$ to a greater extent in prenatal than in postnatal cerebellum, is reminiscent of other rat and avian systems in which CK activity parallels growth and division [47, 48].

In the rabbit kidney, the response to vitamin D metabolites is similar to that in the cerebellum. At −6 days to +3 days, $24,25(OH)_2D_3$ but not $1,25(OH)_2D_3$ increases CKBB activity. At 15 days, only $1,25(OH)_2D_3$ increases CKBB activity while at 8–9 days both metabolites stimulate enzyme activity.

In parallel experiments using the rabbit epiphysis, there is an increase in CKBB activity caused only by $24,25(OH)_2D_3$, throughout the age range tested (−3 to +15 days). $1,25(OH)_2D_3$ does not affect enzyme activity in this age range.

RAT KIDNEY CULTURES

The influence of $24,25(OH)_2D_3$ on the development of responsiveness to $1,25(OH)_2D_3$

Cell cultures from kidneys of 1-week-old rats respond by both increased CKBB specific activity and increased DNA synthesis only to $24,25(OH)_2D_3$ (Fig. 4), whereas cell cultures prepared from kidneys of 5-week-old rats respond only to $1,25(OH)_2D_3$. As found *in vivo* (Fig. 1), an intermediate stage occurs; cell cultures from kidneys of 3-week-old rats respond to both metabolites.

When cells were treated daily with either vehicle or $1,25(OH)_2D_3$, no effect on their pattern of responsiveness to addition of either metabolite is observed at any age, in terms of either increased CK activity (Fig. 5), or augmented DNA synthesis (Fig. 6). However, chronic treatment with $24,25(OH)_2D_3$ causes precocious development of responsiveness to $1,25(OH)_2D_3$ in cell cultures from kidneys of 1-week-old rats (Figs 5 and 6). Similarly, in cell cultures from kidneys of 3-week-old rats, $24,25(OH)_2D_3$ treatment increases the responsiveness to $1,25(OH)_2D_3$ and abolishes the responsiveness to $24,25(OH)_2D_3$ (Figs 5 and 6). In cell cultures from kidneys of 5-week-old rats, $24,25(OH)_2D_3$ no longer has any effect on hormonal responsiveness (Figs 5 and 6). Daily treatment with PTH has no effect on the responsiveness of cultures from kidneys of 1-week-old rats. However, combined treatment with $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ caused the loss of responsiveness to $24,25(OH)_2D_3$ in cultures from kidneys of 3-week-old rats, but did not affect responsiveness in cell cultures prepared from kidneys of 5-week-old rats.

DOES $24,25(OH)_2D_3$ PLAY A ROLE IN NORMAL DEVELOPMENT?

The responsiveness to $24,25(OH)_2D_3$ in non-skeletal tissues (such as kidney and cerebellum), predominantly during early development, suggests that this vitamin D metabolite should be tested for a

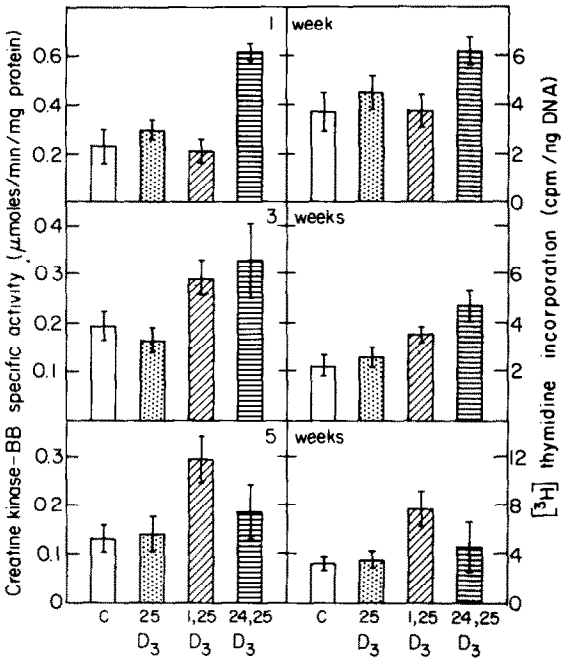

Fig. 4. Age-dependent responsiveness to vitamin D metabolites of renal cell cultures. Kidneys from 1-, 3- and 5-week-old rats were excised, minced and digested in 0.25% trypsin–EDTA for 5 min. The released cells were discarded and the remaining fragments redigested for 2 additional 10-min periods in 0.25% trypsin-EDTA. Released cells were seeded (300,000 cells/35 mm culture dish) in BGJ medium (Maagar, Kibbutz Beit Haemek, Israel) modified to contain 1 mM Ca_{2+} and supplemented with 10% fetal calf serum. Cells were grown for 6–12 days until they reached confluence and were treated with 12 nM of either $25(OH)_2D_3$ ($25D_3$), $1,25(OH)_2D_3$ ($1,25D_3$) or $24,25(OH)_2D_3$ ($24,25D_3$) for 24 h. CKBB activity was measured as described in the legend to Fig. 1. [^{3}H]Thymidine incorporation into DNA during the last 2 h of the incubation period was assayed as described previously [25]. The results are means ± SE for $n = 9$–12 (Sömjen *et al.*, [53].

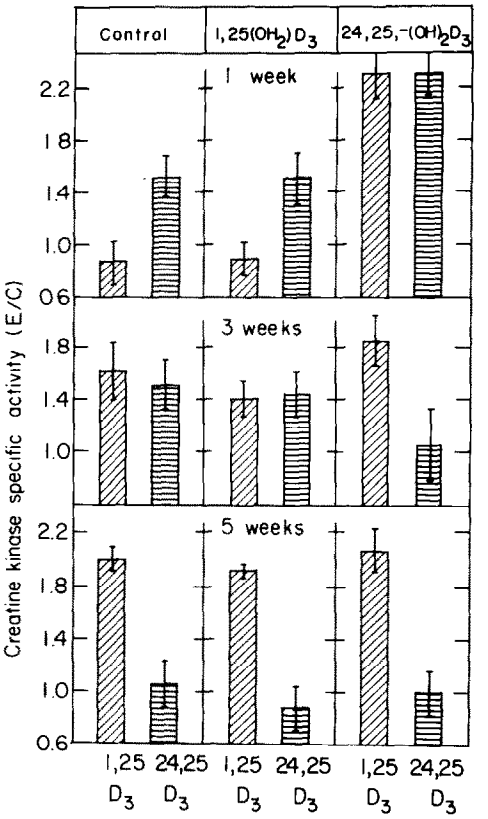

Fig. 5. The effect of chronic treatment with either vehicle (control), $1,25(OH)_2D_3$, ($1,25D_3$) or $24,25(OH)_2D_3$ ($24,25D_3$) on the responsiveness of renal cell cultures to vitamin D metabolites by increased CKBB activity. Chronic treatment of cells was carried out from day 1 of culture; 12 nM $24,25(OH)_2D_3$, 1.2 nM $1,25(OH)_2D_3$ or 10 μl/ml of 20% ethanol in saline (vehicle) was added to the medium daily, for 1 week. Two days later, the responsiveness to either hormone was measured. Cells were cultured and treated as in the legend to Fig. 4. CKBB was extracted and assayed as described in the legend to Fig. 1. Results are expressed as experimental divided by control means ± SE for $n = 6$–9 (Sömjen *et al.*, [53]).

role in this process, not only in kidney and cerebellum, but perhaps in other organs and in species other than rat and rabbit.

We have demonstrated [41] a correlation between the type and concentrations of renal binding proteins for vitamin D metabolites and response to these metabolites by an increase in creatine kinase activity, as well as a correlation between enzyme induction and stimulation of DNA synthesis in several organs [49]. This suggests that the increase in CKBB activity caused by the two vitamin D metabolites at different stages of development is mediated by the parallel, age-related, changes in the concentration of specific binding proteins for the metabolites, each of which may have a specific role during development. In the postnatal development of the intestine, the increase in calcium binding protein in response to $1,25(OH)_2D_3$ starts only from day 18 [35]. No data have yet been reported on possible changes in intestinal mucosa in younger animals caused by $24,25(OH)_2D_3$.

The results obtained with kidney cell cultures (Figs 4–6) in which we can accelerate the maturation of the kidney by chronic treatment with $24,25(OH)_2D_3$ but not $1,25(OH)_2D_3$ (Figs 5, 6) provides data for the general hypothesis that $24,25(OH)_2D_3$ acts in kidney, and in other tissues, as a maturation factor, possibly by induction of the development of receptors to $1,25(OH)_2D_3$, accompanied by down-regulation of its own receptors. This concept has been previously investigated in the pig kidney cell line (LLC-PK1), human skin fibroblasts and human mammary cancer cells (MCF-7) [50]. In these cells, $24,25(OH)_2D_3$ caused an increase of 200–400% in the number of $1,25(OH)_2D_3$ receptors without altering their affinity. The increase takes place within 16–20 h of treatment and is partially dependent on RNA synthesis. Moreover, in chick intestine, $24,25(OH)_2D_3$ allosterically modulates the binding of $1,25(OH)_2D_3$ to its chromatin receptor [51],

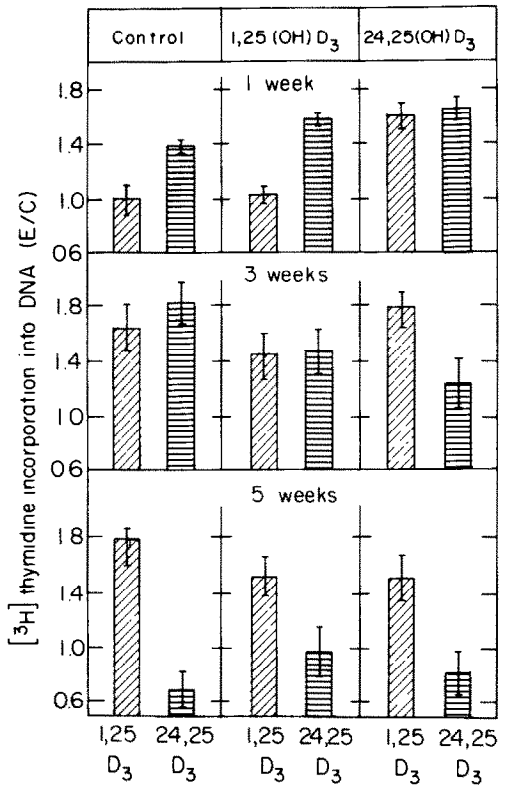

Fig. 6. The effect of chronic treatment with either vehicle (control), $1,25(OH)_2D_3$ ($1,25D_3$) or $24,25(OH)_2D_3$ ($24,25D_3$) on the responsiveness of renal cell cultures to vitamin D metabolites by increased DNA synthesis. Cells were cultured and assayed as described in the legend to Fig. 4. Chronic treatment was described in the legend to Fig. 5 and DNA synthesis was analyzed as described in Fig. 1. Results are expressed as experimental divided by control means ± SE for $n = 6$–9 (Sömjen *et al.*,[53]).

separately from its own specific binding domain in chick intestinal chromatin. This specific binding of $24,25(OH)_2D_3$ is independent of the $1,25(OH)_2D_3$ receptor [52].

Acknowledgements—We thank Mrs Esther Berger for skilled technical assistance and Mrs Rona Levin for efficient processing of the manuscript. This work was supported in part by a grant from the Rockefeller Foundation, New York. AMK is the incumbent of the Joseph Moss Professorial Chair in Molecular Endocrinology at the Weizmann Institute of Science.

REFERENCES

1. Henry H. L. and Norman A. W.: Vitamin D: metabolites and biological action. *Ann. Rev. Nutr.* **4** (1984) 493–520.
2. Tsai H. C. and Norman A. W.: Evidence for a cytoplasmic receptor for 1,25-dihydroxyvitamin D_3 in the intestinal mucosa. *J. biol. Chem.* **248** (1973) 5675–5697.
3. Lawson D. E. M. and Wilson P. W.: Intranuclear localization and receptor proteins for 1,25-dihydroxycholecalciferol in chick intestine. *Biochem. J.* **144** (1974) 573–583.
4. Zerwekh J. E., Lindell T. J. and Haussler M. R.: Rapid enhancement of chick intestinal DNA dependent RNA polymerase II activity by $1\alpha,25$-dihydroxyvitamin D_3 *in vitro*. *J. biol. Chem.* **251** (1976) 2388–2394.
5. Fraser D. R.: Regulation of the metabolism of vitamin D. *Physiol. Rev.* **60** (1980) 551–613.
6. Wasserman R. H. and Taylor T. J.: Vitamin D-induced calcium binding protein in chick intestinal mucosa. *Science* **152** (1966) 791–793.
7. Haussler M., Nagode L. and Rasmussen H.: Induction of intestinal brush border alkaline phosphatase by vitamin D and identity with Ca ATPase. *Nature, Lond.* **228** (1970) 1199–1305.
8. Wilson P. and Lawson D.: Incorporation of [^{3}H]leucine into an actin-like protein in response to 1,25-dihydroxycholecalciferol in chick intestinal brush borders. *Biochem. J.* **73** (1978) 627–631.
9. Raisz L. G., Trummel C. L., Holick M. F. and Deluca H. F.: 1,25 Dihydroxycholecalciferol, a potent stimulator of bone resorption in tissue culture. *Science* **175** (1972) 768–769.
10. Brommage R. and Newman W. F.: Mechanism of mobilization of bone mineral by 1,25-dihydroxyvitamin D_3. *Am. J. Physiol.* **237** (1979) E113–E120.
11. DeLuca H. F.: Some new concepts emanating from a study of the metabolism and function of vitamin D. *Nutr. Rev.* **38** (1980) 169–182.
12. Ornoy A., Goodwin D., Noff D. and Edelstein S.: 24,25-Dihydroxyvitamin D is a metabolite of vitamin D essential for bone formation. *Nature, Lond.* **276** (1978) 517–519.
13. Endo H., Kiyoki M., Kawashima K., Naruchi T. and Hashimoto Y.: Vitamin D_3 metabolites and PTH synergistically stimulate bone formation of chick femur *in vitro*. *Nature, Lond.* **286** (1980) 262–265.
14. Malluche H. H., Henry H., Meyer Saballek W., Sherman S., Massry S. G. and Norman A. W.: Effects and interactions of $24,25(OH)_2D_3$ and $1,25(OH)_2D_3$ on bone. *Am. J. Physiol.* **238** (1980) E494–E496.
15. Henry H. L. and Norman A. W.: Vitamin D: two hydroxylated metabolites are required for normal chick egg hatchability. *Science* **201** (1978) 835–837.
16. Elaroussi M. A., Forte L. R. and Biellier H. V.: Reproduction in quail: biological activity of vitamin D_3 and its metabolites. *J. Bone Mineral Res.* **1** (Suppl. 1) (1986) Abstr. 393.
17. Merke J. and Norman A. W.: Evidence for $24(R)25(OH)_2D_3$ receptor in the parathyroid gland of the rachitic chick. *Biochem. biophys. Res. Commun.* **100** (1981) 551–558.
18. Corvol M. M., Ulmann A. and Garabedian M.: Specific nuclear uptake of 24,25 dihydroxycholecalciferol, a vitamin D_3 metabolite biologically active in cartilage. *FEBS Lett.* **116** (1980) 273–276.
19. Sömjen D, Sömjen G. J., Weisman Y. and Binderman I.: Evidence for 24,25-dihydroxycholecalciferol receptors in long bones of newborn rats. *Biochem. J.* **204** (1982) 31–36.
20. Sömjen D., Sömjen G. J., Harell A., Mechanic G. L. and Binderman I.: Partial characterization of a specific high affinity binding macromolecule for 24R,25 dihydroxyvitamin D_3 in differentiating skeletal mesenchyme. *Biochem. biophys. Res. Commun.* **106** (1982) 644–651.
21. Harel S., Watanabe K., Linke I. and Schain R. J.: Growth and development of the rabbit brain. *Biol. Neonate.* **21** (1972) 381–399.
22. Sömjen D., Binderman I. and Weisman Y.: The effects of 24R,25 dihydroxycholecalciferol and 1,25 dihydroxycholecalciferol on ornithine decarboxylase activity and on DNA synthesis in the epiphysis and diaphysis of rat bone and in the duodenum. *Biochem. J.* **214** (1983) 293–298.

23. Sömjen D., Weisman Y., Binderman I. and Kaye A. M.: Stimulation of creatine kinase BB by 24R,25 dihydroxycholecalciferol in rat tissues. *Biochem. J.* **219** (1984) 1037–1041.
24. Sömjen D., Weisman Y., Berger E., Fine N., Kaye A. M. and Binderman I.: A comparison of the responses to 24R,25$(OH)_2D_3$ and 1,25$(OH)_2D_3$ by developing skeletal tissue. In *Vitamin D. A Chemical, Biochemical and Clinical Update* (Edited by A. W. Norman, K. Schaefer, H. G. Gilgoleit and D. V. Herroth). Walter de Gruyter, Berlin (1985) pp. 284–293.
25. Binderman I. and Sömjen D.: 24R,25-Dihydroxycholecalciferol induces the growth of chick cartilage *in vitro*. *Endocrinology* **115** (1984) 430–432.
26. Sömjen D., Kaye A. M. and Binderman I.: 24R,25 Dihydroxyvitamin D stimulates creatine kinase BB activity in chick cartilage cells in culture. *FEBS Lett.* **167** (1984) 281–284.
27. Fraser D. R. and Kodicek E.: Unique biosynthesis by kidney of a biologically active vitamin D metabolite. *Nature, Lond.* **228** (1970) 764–766.
28. Haussler M. R. and McCain T. A.: Basic and clinical concepts related to vitamin D metabolism and action. *N. Engl. J. Med.* **297** (1977) 974–983.
29. Holick M. F., Schones H. K., DeLuca H. F., Gray R. W., Boyle I. and Suda T.: Isolation and identification of 24,25 dihydroxycholecalciferol, a metabolite of vitamin D_3 made in the kidney. *Biochemistry* **11** (1972) 4251–4255.
30. Chandler J. S., Pike A. W. and Haussler M. R.: 1,25 dihydroxyvitamin D_3 receptors in rat kidney cytosol. *Biochem. biophys. Res. Commun.* **90** (1979) 1057–1063.
31. Christakos S. and Norman A. W.: Studies on the mode of action of calciferol. XXIX. Biochemical characterization of 1,25 dihydroxyvitamin D_3 receptors in chick pancreas and kidney cytosol. *Endocrinology* **108** (1981) 140–149.
32. Colston K. and Feldman D.: Nuclear translocation of the 1,25 dihydroxycholecalciferol receptor in mouse kidney. *J. biol. Chem.* **255** (1980) 7510–7513.
33. Simpson R. U., Franceschi R. T. and DeLuca H. F.: Characterization of a specific high affinity binding macromolecule for 1,25 dihydroxyvitamin D_3 in cultured chick kidney cells. *J. biol. Chem.* **255** (1980) 10,160–10,166.
34. Norman A. W., Roth J. and Orci L.: The vitamin D endocrine system: steroid metabolism, hormone receptors and biological response. *Endocr. Rev.* **3** (1982) 331–366.
35. De Lorme A. C., Marche P. and Garel J. M.: Vitamin D-dependent calcium binding protein changes during gestation, prenatal and postnatal development in rats. *J. dev. Physiol.* **1** (1979) 181–185.
36. Rhoten W. B. and Christakos S.: Immunocytochemical localization of vitamin D dependent calcium binding protein in mammalian nephron. *Endocrinology* **109** (1981) 981–983.
37. Roth J., Brown D., Norman A. W. and Orci L.: Localization of the vitamin D dependent calcium binding protein in mammalian kidney. *Am. J. Physiol.* **243** (1982) F243–F252.
38. Taylor A. M., McIntosh J. E. and Bourdeau J. E.: Immunochistochemical localization of vitamin D dependent calcium binding protein in renal tubules of rabbits, rats and chicks. *Kidney Int.* **21** (1982) 765–773.
39. Wasserman R. H. and Fullmer C. S.: Calcium transport proteins, calcium absorption and vitamin D. *Ann. Rev. Physiol.* **45** (1983) 375–390.
40. Wasserman R. H., Brindak M. E., Meyer S. A. and Fullmer C. S.: Evidence for multiple effects of vitamin D_3 on calcium absorption: response of vitamin D-deficient chick, with or without partial vitamin D_3 repletion to 1,25 dihydroxyvitamin D_3. *Proc. natn. Acad. Sci. U.S.A.* **79** (1982) 7939–7943.
41. Sömjen D., Weisman Y., Berger E., Earon Y., Kaye A. M. and Binderman I.: Developmental changes in the responsiveness of rat kidney to vitamin D metabolites. *Endocrinology* **118** (1986) 354–359.
42. Fleck C. and Braünlich H.: Kidney function after unilateral nephrectomy. *Exp. Path.* **25** (1984) 3–18.
43. Jande S. S., Tolnai S. and Lawson D. E.: Immunohistochemical localization of vitamin D dependent calcium binding protein in duodenum, kidney, uterus and cerebellum of chickens. *Histochemistry* **71** (1981) 99–116.
44. Roth J., Baetnes D., Norman A. W. and Garcia Segura L. M.: Specific neurons in chick central nervous system stain with an antibody against chick intestinal vitamin D dependent calcium binding protein. *Brain Res.* **222** (1981) 452–457.
45. Taylor A. N.: Chicken brain calcium binding protein: response to cholecalciferol and some developmental aspects. *J. Nutr.* **107** (1977) 480–486.
46. Sonnenberg J., Luine V. H., Krey L. and Christakos S.: Vitamin D_3(1,25$(OH)_2D_3$) results in increased choline acetylase activity in specific brain nuclei. In *Vitamin D. A Chemical, Biochemical and Clinical Update* (Edited by A. W. Norman, K. Schaefer, H. G. Gilgoleit and D. V. Herroth). Walter de Gruyter, Berlin (1985) pp. 117–118.
47. Reiss N. A. and Kaye A. M.: Identification of the major component of the estrogen induced protein of rat uterus as the BB isozyme of creatine kinase. *J. biol. Chem.* **256** (1981) 1899–1904.
48. Sömjen D., Kaye A. M. and Binderman I.: Stimulation of creatine kinase BB activity by parathyroid hormone and by prostaglandin E_2 in cultured bone cells. *Biochem. J.* **225** (1985) 591–596.
49. Kaye A. M., Reiss N. A., Weisman Y., Binderman I. and Sömjen D.: Hormonal regulation of creatine kinase BB. In *Myocardial and Skeletal Muscle Bioenergetics* (Edited by N. Brautbar), Plenum, New York (1986) pp. 83–101.
50. Costa E. M., Hirst M. A. and Feldman D.: Regulation of 1,25 dihydroxyvitamin D_3 receptors by vitamin D analogs in cultured mammalian cells. *Endocrinology* **117** (1985) 2203–2210.
51. Wilhelm F. and Norman A. W.: 24R,25 Dihydroxyvitamin D_3 regulates 1,25 dihydroxyvitamin D_3 binding to its chick intestine receptor. *Biochem. biophys. Res. Commun.* **126** (1985) 496–501.
52. Wilhelm F., Ross P. F. and Norman A. W.: Specific binding of 24R,25 dihydroxyvitamin D_3 to chick intestinal mucosa: 24R,25 dihydroxyvitamin D_3 is an allosteric effector of 1,25 dihydroxyvitamin D_3 binding. *Archs. Biophys.* (1986) In press.
53. Sömjen D., Weisman Y., Berger E., Harell A., Kaye A. M. and Binderman I.: The role of 24,25$(OH)_2$ vitamin D3 in the maturation of rat kidney. In *Adv. Skeletogenesis* **3** (Edited by S. Hurwitz and J. Sela). Heiliger, Jerusalem (1987). In press.