The site of 25-hydroxycholecalciferol stimulated protein synthesis in the rat kidney

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Summary. Autoradiographs of the kidneys of rachitic rats dosed in vivo with 250 pmoles 25-hydroxycholecaliferol (25-OHD_3) and ^{3}H-leucine showed increased grain counts in portions of proximal renal tubules. On incubation of kidney slices the synthesis of only 1 cytosol protein was found to be stimulated by the steroid. On disc gel electrophoresis it had the characteristics of renal calcium binding protein.

There is an increased awareness of the action of vitamin D or its metabolites on the renal tubule, quite apart from the renal tubule's action in modifying the cholecalciferol molecule. There is evidence that vitamin D increases the renal reabsorption of calcium[1,2] and in this connection the reports of a vitamin-D-dependent calcium binding protein (Ca BP) in the chick[3] and rat[4] kidney may be of significance. We have investigated the effect of vitamin D on protein synthesis in the rat kidney using radioactive leucine as a marker and report here our preliminary results.

Materials and methods. All experiments were performed in duplicate. Rachitic rats were reared on a semi-synthetic high calcium low phosphorus diet and used at approximately 9 weeks age (70–90 g b.wt). In the 1st study, batches of 5 animals were given 250 pmoles 25-hydroxycholecaliferol (25-OHD_3) i.v. and killed at 0, 4, 8, 18, 30 h after dosing. 2 h before death, each animal was given ^{3}H-leucine (5 µCi/g b.wt) by i.p. injection. Those killed at 0-time served as controls. Immediately after death, the kidneys were fixed in 5% formalin and 2-µm sections were prepared on subbed slides for autoradiography, using Ilford K5 emulsion. Radiographs were developed after 3 week' exposure, and stained lightly with haematoxylin.

In a 2nd experiment, kidney cortex slices (300 mg) from 4 rachitic rats were incubated for 4 h in 10 ml of leucine-free minimum essential medium (Gibco-Biocult), 20 mM HEPES buffer (Sigma, London), penicillin and streptomycin (1 mg/ml of each), 10 µCi ^{14}C-leucine at pH 7.5. The flasks were gassed with 5% CO_2 in O_2 at the start of incubation and after 2 h. Simultaneously a similar procedure was carried out with kidney slices from 4 rachitic rats each predosed with 250 moles 25-OHD_3 34 h previously, and using ^{3}H-leucine in the incubation medium. The rationale for this was that any influence of 25-OHD_3 on protein synthesis would be reflected in an altered incorporation of radioactive leucine into protein during its formation. Since the ^{14}C- and ^{3}H-leucine can be readily distinguished, it would be possible, following protein fractionation, to decide whether the pattern of leucine incorporation differed in the tissue from animals pretreated with 25-OHD_3 from that in controls.

At the end of incubation, the slices were removed and appropriate ones pooled. They were washed 3 times in ice-cold saline and gently homogenised in 1.5 ml 40 mM NaCl, 10 mM Tris pH 7.5 with 10 strokes of a Teflon-glass homogeniser. The homogenate was centrifuged for 30 min at 50,000 × g and the supernatant collected. 50-µl portions of the ^{14}C- and ^{3}H-labelled supernatants were pooled and fractionated by disc gel electrophoresis[5]. A 2nd portion was treated with sodium dodecyl sulphate (SDS) and fractionated by SDS gel electrophoresis.

The gels were scanned in a Joyce-Loebl UV scanner, and then sliced accurately into 1-mm slices using a Yeda gel-slicer. The slices were solubilised in Nuclear (Chicago) solubuliser, and toluene-ethoxyethanol-diphenyloxazole scintillation cocktail added before counting for 20 min in a Packard Tricarb Scintillation Spectrometer. Quenching correction was calculated by means of an automatic external standardisation and correlation curves for combined ^{3}H and ^{14}C. The ratio of ^{3}H- and ^{14}C-radioactivity was calculated for each slice and related to its position on the gel, with any increased ratio indicating a change in protein synthesis due to 25-OHD_3. The technique was also used to look for evidence of vitamin-D-induced protein synthesis in mitochondrial and microsomal fractions, carefully prepared using standard ultracentrifugation techniques and giving satisfactory morphology on electron microscopy. The particles were solubilized in 2 M urea followed by SDS and applied to gels which were scanned, sliced and counted in the manner described.

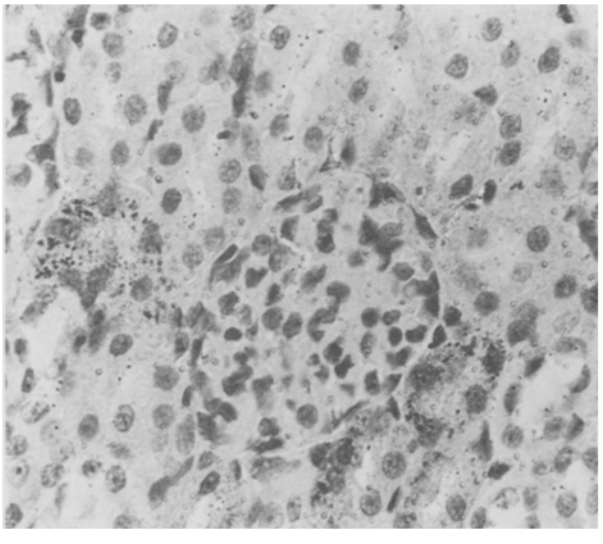

Fig. 1. Autoradiograph of renal cortex of rat dosed 30 h previously with 250 pmoles 25 OHD_3. × 50.

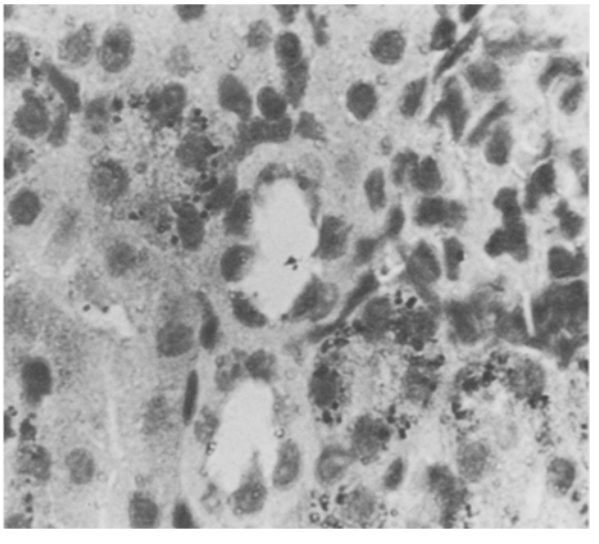

Fig. 2. Autoradiograph from a rat treated as in figure 1. × 80.

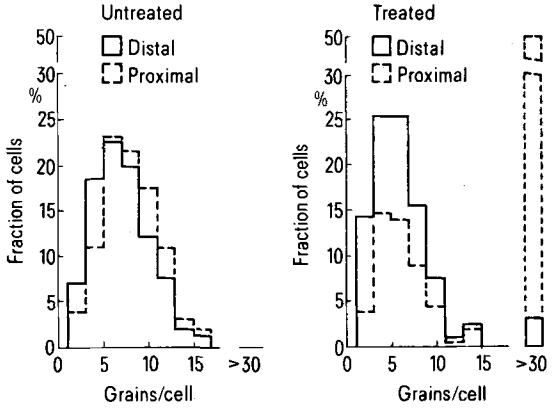

Fig. 3. Distribution of grain counts in proximal and distal convoluted tubule cells in untreated and treated rats.

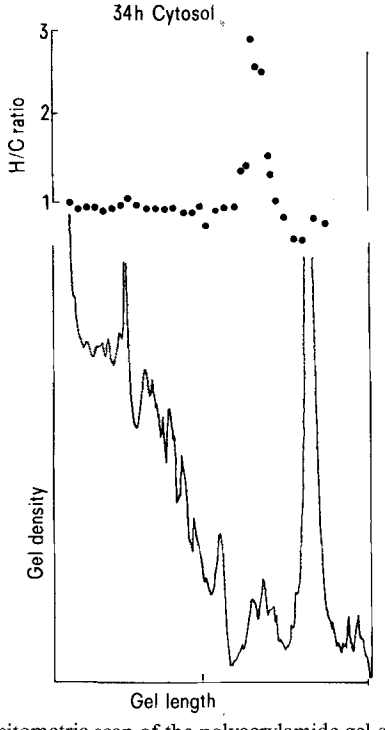

Fig. 4. Densitometric scan of the polyacrylamide gel electrophoresis of the cytosol preparation, with the $^3H/^{14}C$ ratio for the corresponding 1 mm slices shown above.

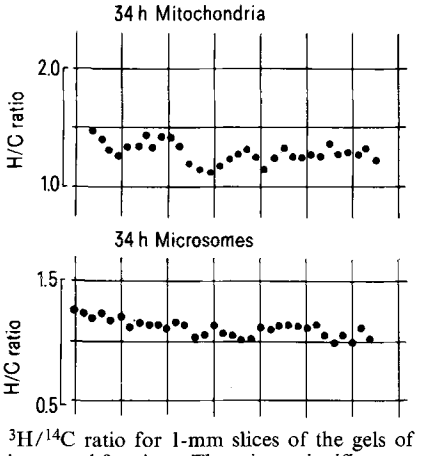

Fig. 5. The $^3H/^{14}C$ ratio for 1-mm slices of the gels of mitochondrial and microsomal fractions. There is no significant variation.

Results and discussion. The autoradiographs indicate that there is an increase in the rate of synthesis of protein in a portion of the proximal tubular cells 18–30 h after a dose of 25-OHD_3. The sections from animals predosed up to and including 18 h were identical to those from controls, but the autoradiographs from the animals dosed 30 h before death showed clusters of tubule cells marked out by greatly increased numbers of grains (figures 1 and 2). The cells were identifiable as proximal tubule type on the basis of standard morphology, including the brush border which was readily seen with phase contrast microscopy. However, there were many other portions of proximal tubule in which the number of grains were not increased. This is illustrated quantitatively in figure 3, which shows the distribution of grain counts carried out in the region of glomeruli in control and 30-h kidneys.

In order to investigate which protein or protein were represented in the areas of increased leucine incorporation, the 2nd experiment was carried out. Incubating kidney cortex slices from treated and control animals with medium containing ^3H- and ^{14}C-leucine respectively demonstrated enhanced protein synthesis in the cytosol fraction of tissue from treated animals. This was evidenced by a marked increase in the $^3H/^{14}C$ ratio, which appeared as a sharp peak in 1 zone only (figure 4) implying that 25-OHD_3 promoted synthesis of only one cytosol protein in the kidney. The peak had the same relative mobility as rat kidney CaBP run in an identical system. Using the procedure with SDS gels, the peak corresponded with a protein of 25,000 daltons and it is therefore likely that the protein in question is the rat kidney calcium binding protein (mol. wt 28,000). The fact that the protein synthesis demonstrated by the autoradiography is confined to the proximal tubules, while we have confirmed the presence of a vitamin-D-dependent protein with the electrophoretic properties of CaBP, is of interest, since micropuncture studies[6] have shown that in the rat kidney during the process of reabsorption the greatest calcium load is handled by the proximal tubule cells, possibly paralleling the situation in the gut where calcium binding protein appears at the site of maximal calcium absorption[7]. There is good evidence that in the chick kidney CaBP is found in the distal tubule[8] but data concerning tubular reabsorption of calcium is not available.

In the mitochondrial and microsomal particles, the solubilisation and electrophoresis yielded excellent resolution but after slicing there was no variation in the $^3H/^{14}C$ ratio (figure 5) implying that no vitamin-D-dependent protein synthesis was taking place in these fractions, at least at the time interval employed. Experiments to examine the surface membranes in the same way are to be undertaken.

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