Immunoreactivity of PTH-binding in intact bovine kidney tissue and cultured cortical kidney cells indicative for specific receptors

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Abstract. Localization of PTH-binding sites has been examined in intact kidney sections and cultured cells derived from bovine kidney cortex. Tissue sections were incubated with 10^{-7}M bovine PTH (1-84) for 2 h, cells for 15 min, at 37°C. Visualization of PTH-binding was achieved by immunocytochemistry using a carboxy-terminal specific anti-PTH antiserum (S 478). For control, cell culture incubations were performed applying competitively 10^{-7}M bovine PTH (1-84) and a 10-fold excess of synthetic 1-34 PTH fragment, not antigenic for S 478. This resulted in a lack of staining. PTH-binding was found in all cells of the proximal and the distal tubule, and with less intensity in the thick ascending limb of Henle’s loop. In collecting ducts a PTH specific staining was also present, which was confined to single cells localized between others without PTH binding sites. No staining was seen in glomerula, the thin limb of Henle’s loop, in blood vessels, and in connective tissue. The data suggest that large parts of the nephron contain PTH-binding sites, although in different amounts. This is in agreement with the numerous actions of PTH in the kidney. In the collecting segment a distinct cell-to-cell difference was disclosed indicative for different functional states or cellular heterogeneity.

Parathyroid hormone (PTH) action on the kidney causes enhanced calcium re-absorption, phosphaturia, bicarbonaturia, and enhanced 25-hydroxycholecalciferol-1-hydroxylase activity. Exact localization of PTH binding sites in the different segments of the nephron is still under debate. Specific receptors for PTH have been demonstrated biochemically by Zull et al. (1977) in membrane preparations of bovine kidney cortex and by Teitelbaum et al. (1984) in an established cell line of cortical kidney cells. Histological assessment of PTH receptors has been performed by Nordquist & Palmieri (1974), by Stumpf et al. (1980) and Rouleau et al. (1986) who applied immunocytochemistry and autoradiography. Additionally, our group reported PTH-binding to cultured cells from bovine kidney cortex using a biotinylated PTH analogue (Dietel et al. 1985; Niendorf et al. 1986). These studies disagree with regard to the morphological localization of PTH-binding to the tubule system. In the present paper immunocytochemical assessment of PTH-binding to intact kidney tissue and cultured kidney cells is presented. The results provide new insights on PTH binding pattern in the nephron and confirm in part results obtained by other groups.

Material and Methods

Tissue processing

Bovine kidneys were obtained from a local slaughter house. Small (approx 1 mm³) tissue specimens were cut from the cortex and placed immediately into Hepes buffered Hank’s balanced salt solution. The pieces were incubated with 10^{-7}M bovine (b) PTH (1-84), a generous gift from Dr Terpin, Hormonchemie, Munich, or with plain diluent for 2 h at 37°C. Subsequently, the tissue was washed several times, fixed in bouin’s solution for 1 h, embedded in paraffin and processed for immunocytochemistry (see below). To exclude the pres-
ence of endogenous PTH controls were performed without PTH incubation.

Cell culture technique
Cultured cells from bovine kidney cortex were gained as described previously in detail (Dietel et al. 1986; Niendorf et al. 1986). Kidney cells, grown on untreated glass slides, were incubated for 15 min with $10^{-7}$ M of b-PTH 1–84. After extensive washing fixation was done in 1:5 (vol/vol) Bouin solution/PBS. To prove specificity of the PTH 1–84 binding the cells were pre-incubated for 24 h in a serum-free culture medium and subsequently incubated simultaneously with $10^{-7}$ M b-PTH 1–84 and a 10-fold molar excess of a synthetic b-PTH 1–34.

Immunocytochemistry
Sections from paraffin blocks and cultured cells were stained for PTH using a highly specific anti PTH-antibody (S 478, a gift from Dr Hesch, Hannover, FRG). It is directed against the carboxy-terminal region of the PTH molecule (Dietel & Hölzel 1983), thus detecting a 1–84 PTH, but not a 1–34 PTH fragment as confirmed by dot-blots on nitrocellulose paper. For visualization, the unlabelled antibody method (Sternberger 1979) and the avidin-biotin peroxidase technique (Hsu 1981) were applied. Controls were performed using pre-absorbed primary antibody, substituting primary antibody by normal sheep serum, and by omitting each other step of the staining procedure separately.

Results
Controls
In kidney sections endogenous PTH was excluded when untreated sections were processed for immunocytochemistry resulting in a lack of staining. All other controls showed loss of any specific staining.

PTH binding to intact kidneys
Both methods, PAP and ABC resulted in a staining of comparable intensity. Immunoreactivity cold be detected in the proximal tubule, the thick limb of Henle’s loop, the distal tubule, and the collecting duct (Figs. 1 to 3). The staining intensity varied between the different parts of the nephron. All cells of the proximal tubule stained strongly positive for PTH (Fig. 1). From cell to cell there was no difference in staining intensity. Cells of the thick ascending limb of Henle’s loop and the distal tubule also stained positive, however, with reduced intensity (Fig. 2). In contrast, only single cells within the collecting duct expressed PTH immunoreactivity (Fig. 3). Noteworthy, this was stronger than in the other parts of the tubule system. No staining was seen in glomerula, the thin limb of the loop of Henle, in blood vessels and in connective tissue.

Concerning the cellular distribution pattern of

![Immunocytochemical staining of PTH binding in proximal (x) and distal tubules (x 300).](image)

Fig. 1.
Fig. 2.
Immunocytotoxic staining of PTH binding in distal tubules.
Note the non-stained glomerulum at the left (× 100).

PTH-binding immunostaining was observed at the membrane and in the cytoplasm. The nucleus was free of the reaction product. Differences with respect to apical, basal or membrane associated localization of the staining product were not present.

*PTH-binding of cultured kidney cells*
Monolayer cultures of kidney cortex cells showed a staining reaction in 30% of the cells (Fig. 4). The immunoreaction was again excluded from the nucleus with a predominance in the perinuclear region. After a 24 h pre-incubation in serum free medium competition of b-PTH 1–84 with a 10-fold excess of a synthetic b-PTH 1–34 reduced the staining reaction to background level when the carboxy-terminal specific antiserum was applied.

Fig. 3.
Immunocytotoxic staining of PTH binding in collecting ducts with distinct cell-to-cell differences
(left: × 150, right: × 1000)
Discussion

In the paper we present the morphological demonstration of immunoreactivity specific for the carboxy-terminal region of PTH. The value of the methodological approach has been documented in a number of papers (Dietel & Hölzel 1983; Arps et al. 1986) investigating the secretory and storage pattern of parathyroid glands. We interpret PTH-specific immunoreactivity as indicative for PTH binding to specific receptors, because in cell culture binding experiments staining could be reduced to background level by competitive incubation of b-PTH 1–84 with b-PTH 1–34, a sequence not reacting with the used anti PTH-antibody.

The first report of the morphological visualization of PTH-binding in kidney target cells (Nordquist & Palmieri 1974) used the immunofluorescence technique. Rao et al. (1983) incubated deparaffinized sections of fixed decalcified rat radii with b-PTH, which was subsequently visualized by means of immunocytochemistry. Both groups deduced from their experiments the specific staining of receptors. Stumpf et al. (1980) who as well determined PTH receptors exclusively in proximal tubules of rat kidney stated that this result does not exclude other sites of PTH-binding in the kidney. Biochemical data gained by micropuncture technique (Morel et al. 1983) supported a more general distribution in the nephron. In the rabbit kidney PTH activates adenyl-

ate cyclase to different extends in the proximal tubule, the thick ascending limb of Henle's loop, the distal tubule, and the collecting ducts. Our results confirm this pattern of PTH action by visualization of specific receptors in the described segments of the nephron. They are consistent with those obtained by Rouleau et al. (1986) with the exception of PTH-binding to glomerula. Regarding the cellular distribution we could not differentiate between apical or basal localization of the immunoreaction, which may be explained by PTH internalization during the 2 h experimental period.

In addition, this study discloses cell-to-cell differences of PTH-binding in the collecting ducts. This may be due to different functional states of the cells or heterogeneous cell populations. Autoradiographic studies (Rouleau et al. 1986) discriminate grain densities in different parts of the nephron, however, they did not describe differences in staining intensity from cell to cell. Our finding of PTH-binding to single cells of the distal part of the nephron indicates a highly differentiated and specialized organization of that nephron segment. Functional meaning of this finding remains to be investigated. Since biochemical methods fail to elucidate whether PTH binding cells are distributed homogeneously or heterogeneously along the nephron morphological receptor studies are a helpful tool to answer this question.
References


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