J. steroid Biochem. Vol. 27, No. 4–6, pp. 953–961, 1987
Printed in Great Britain.

0022-4731/87 $3.00 + 0.00

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

28. Steroid Hypertension and Renal Function

BINDING AND ACTION OF ALDOSTERONE, DEXAMETHASONE, 1-25(OH)$_2$D$_3$, AND ESTRADIOL ALONG THE NEPHRON

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Summary—The localization of specific binding sites of four steroids—aldosterone, dexamethasone, 1-25-dihydroxycholecalciferol and estradiol—is described along the nephron. This localization has been determined by using an autoradiographic method on dry films, applied to intact microdissected tubular segments.

Aldosterone binds specifically to nuclei of the distal and cortical collecting tubule. No specific labeling was observed in the cytoplasm. This localization corresponds to the known sites of action of aldosterone on sodium reabsorption. Specific nuclear binding of dexamethasone is present all along the tubule except the proximal tubule. In this latter part of the nephron, a specific cytoplasmic labeling is observed, in the absence of nuclear labeling. This cytoplasmic binding could correspond to physiological effects in this structure, via non-genomic mechanisms.

1-25(OH)$_2$D$_3$ nuclear binding is located mainly in the loop of Henle and medullary collecting tubule, which are the sites of synthesis of calcium-binding proteins. No specific binding of estradiol is present in any part of the nephron.

The goal of this section is to give to specialists in the field of steroid hormones brief information on the localization of binding sites of some steroids along the nephron, and on what is known about the specific action of these steroids within the kidney.

Since it is probable that most of the readers are more familiar with the molecular behavior of steroids than with the anatomy and physiology of the kidney, I shall begin with a brief overview of the morphology of the nephron. This should convince the readers of the great heterogeneity of this structure, and of the interest in working on isolated, precisely defined parts of the nephron. Then I shall describe the localization and characteristics of binding for four steroids, aldosterone, dexamethasone, 1-25(OH)$_2$ vitamin D$_3$ and estradiol. Most of these results have been obtained on isolated tubular segments, using autoradiographic methods which have been developed in our laboratory. Finally, a summary of the main known action of these hormones on water and solute transport processes along the nephron will be given.

MORPHOLOGY OF THE NEPHRON

The nephron is the functional unit of the kidney [1]. Human kidney contains about one million nephrons, rabbit kidney 200,000, rat kidney 30,000. The nephron consists of a filtration barrier, the glomerulus, followed by the tubule. After ultrafiltration of the blood in the glomerulus, ultrafiltrate flows along the tubule, where reabsorption and secretion processes take place, in order to elaborate the final urine which is excreted into the bladder. The tubule is formed by a succession of epithelia with very different anatomical and functional properties, in particular with regard to electrolyte transport. Indeed, the binding capacity for various hormones and the final action of these hormones on transport processes vary widely according to the different epithelial types along the tubule. Figure 1 illustrates the general organization of the mammalian kidney, and gives a scheme of the nephron, with the localization of each particular tubular type within the kidney.

Roughly, the glomerulus is immediately followed by the proximal tubule (about 80% of renal tubular mass), consisting of a "leaky" epithelium formed of cells with a large "brush-border" on the luminal site [2]. The bulk of filtered water and solutes (in particular sodium) is reabsorbed in the proximal tubule, which is almost entirely located in the superficial part of the kidney (cortex). Proximal tubule is followed by the loop of Henle, a hairpin segment which penetrates into the deep region of the kidney, the medulla. This segment is composed of a descending and an ascending limb (successively thin and thick) which returns to the cortex. It is responsible for the concentration-dilution mechanisms of urine. A short segment, the distal tubule, makes the junction with the collecting system, which consists of a cortical part and a medullary terminal portion. Cells of the loop of Henle, distal and collecting tubules, have no brush-border. Distal and collecting tubular cells consist of tight epithelia, with tight junctions, resembling epithelia of the amphibian bladder. In these segments, final, precise adjustments of the urine composition occur.

From this figure, it appears clearly that experiments on total kidney tissue (particularly by biochemical methods), or even on parts of the kidney, such as cortex or medulla, are performed on a

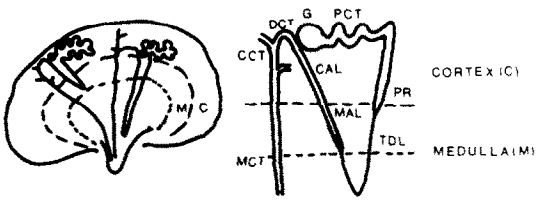

Fig. 1. Left: the general organization of the kidney, with its superficial zone, the cortex (C) which contains mainly glomeruli (G) and proximal convoluted tubules (PCT), but also the thick cortical part of the ascending limb of the loop of Henle (CAL) and the distal and cortical collecting tubules (CCT). The medulla (M) is the deep region of the kidney. It contains the major part of the loop of Henle, thin descending limb (TDL) and the ascending limb in its medullary portion (MAL). Right: a scheme of the nephron (see text for explanation).

mixture of very heterogeneous cells. This precludes any firm conclusion on a precise localization of binding or action of hormones along the nephron. This is why it was necessary to develop methods to obtain data from isolated nephron segments. Physiological results for electrolyte transport by single defined tubules are obtained by *in vivo* micropuncture experiments, or by using tubular segments isolated by microdissection. The obtention of microdissected segments also allows a biochemical approach (by miniaturized methods) or, mainly, autoradiographic studies on hormone binding.

METHODS

Most of the results concerning the localization of steroid binding along the nephron were obtained in our laboratory by autoradiography on isolated tubular segments obtained by microdissection [3]. Kidney pyramids are incubated for 1 h at 30°C in the presence of both collagenase and a tritiated steroid, with or without competitor. Thereafter microdissection is performed at 4°C in the absence of steroid. For autoradiography, isolated segments are transferred on a glass cover-slip and applied in the dark on dry film. Exposure is 2–4 months. Fixation and staining take place after exposure. Number of silver grains per unit surface of cytoplasm or nuclei are determined on enlargement of the photographs. Specific binding is calculated by subtracting silver grain counts after incubation in the presence of an excess cold hormone from those in the presence of labeled hormone alone. We studied dose-dependent binding and the specificity of the binding by competition experiments.

In some cases, miniaturized biochemical methods and HPLC were applied to pools of tubular segments [4]. Induction of RNA synthesis by adrenal steroids was examined on such material by autoradiographs of [^{3}H]uridine incorporation [5, 6]. Experiments were done on animals depleted in the endogenous steroid studied. Figure 1 gives the scheme of a nephron with the different segments studied.

Results concern mainly the localization of aldosterone receptors, and also those of dexamethasone, 1-25-dihydroxyvitamin D_3, and estradiol.

ALDOSTERONE

Figure 2 shows autoradiographs of the binding of tritiated aldosterone in the collecting tubule of rabbits previously depleted in endogenous aldosterone by a low-potassium–high-sodium diet. Silver grains are concentrated on nuclear shadows, and almost no cytoplasmic silver grains are present. The labeling is very well displaced by 200-fold excess unlabeled hormone, assessing the specificity of the nuclear binding.

The profile of nuclear binding for aldosterone has been established along the rat and rabbit nephron [7–9] and is illustrated in Fig. 3. No major difference appears between these two species. At physiological concentration (0.2–2 nM), the distal and cortical collecting tubules are clearly the major sites of specific nuclear binding of aldosterone. Some specific binding is also present, to a lesser degree, in the cortical part of the ascending limb of the loop, and in the medullary collecting duct. No significant binding is observable in the other parts of the nephron, and particularly in the proximal tubule. With increasing concentrations, the binding increases and becomes equivalent in all segments from CAL to MCT. This probably reflects progressive occupancy of lower affinity sites, called type II sites, more specific for glucocorticoids. Biochemical experiments also demonstrate the presence of specific nuclear binding sites in isolated cortical collecting tubule, and their absence in proximal tubule [4]. The fact that binding sites in CCT are about 10 times more numerous (10,000 per cell) than the previous estimates for whole kidney constitutes a further argument to designate the cortical collecting tubule as the most specific target site for aldosterone. *In vivo* experiments, after injection of tritiated aldosterone, give similar results [10].

A striking feature of aldosterone binding in target segment is its almost exclusive nuclear accumulation. This observation was confirmed in CCT, even after incubation at 4°C, where the binding is lower, but remained exclusively nuclear, without cytoplasmic accumulation [11]. Similar results have been reported for other steroid hormones in their target tissue [12, 13].

Other approaches indicate that CCT is the preferential target site for aldosterone. Enzyme induction by this hormone has been shown in CCT. This is the case for citrate synthase [14] and Na–K-ATPase [15]. We shall not detail these aspects, developed in the following sections. Biochemical and autoradiographic methods on isolated tubules

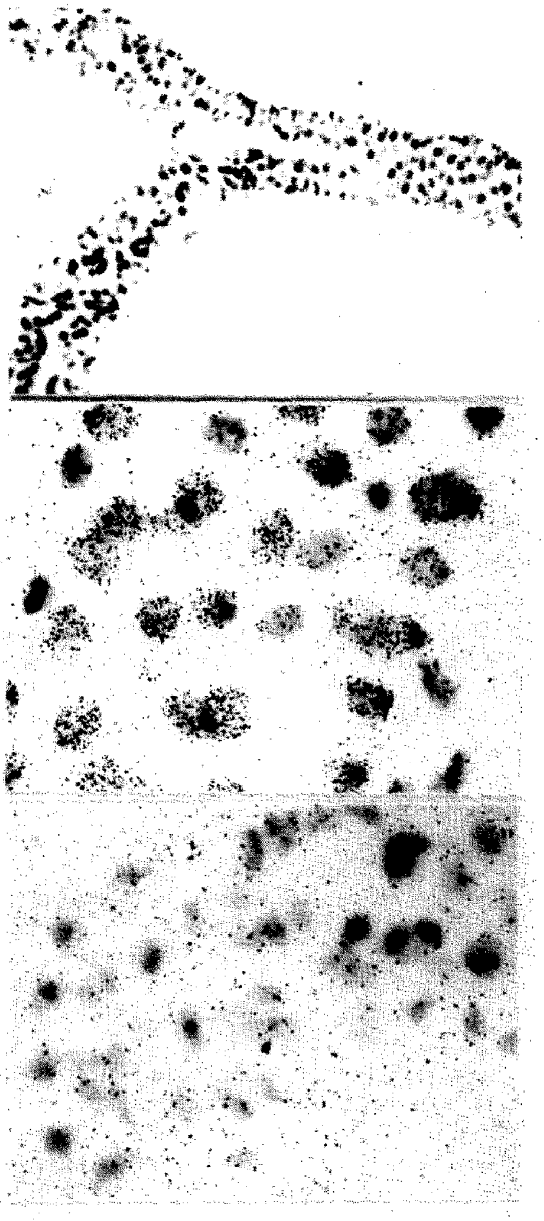

Fig. 2. Specific nuclear binding of aldosterone in cortical collecting tubule. Top: CCT at low magnification. The tubular shape is visible. Silver grains are concentrated on nuclear shadows. Middle: CCT at a higher magnification. The almost exclusive nuclear labeling is evident. Note the heterogeneity of labeling from one nucleus to another. Bottom: In the presence of an excess (×200) unlabeled hormone, the labeling is very well displaced, and not higher than the background, for nuclei as well as cytoplasm. Experiment performed in the rabbit after *in vitro* incubation of kidney pyramids in the presence of 2 nM [^{3}H]aldosterone.

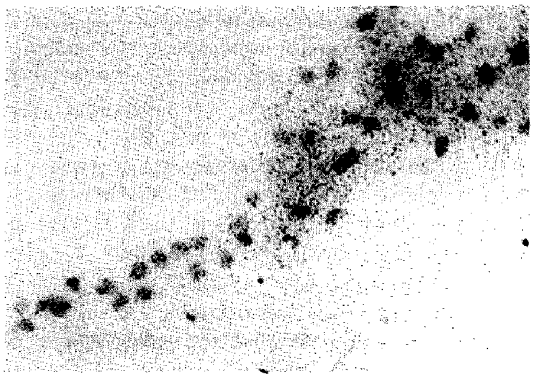

Fig. 4. Autoradiograph of dexamethasone labeling in pars recta and thin descending limb of the loop of Henle. A diffuse labeling, not different in the nuclear shadows and in cytoplasm is present in the terminal, large, part of the pars recta. This segment is followed by a thin segment, the thin descending limb. In this latter segment, silver grains are concentrated on nuclear shadows, with very few grains in cytoplasm. The transition between these two types of tubular segments belonging to the same nephron is very sharp. Experiment performed on the rabbit after *in vitro* incubation of kidney pyramids in the presence of 20 mM [^{3}H]dexamethasone.

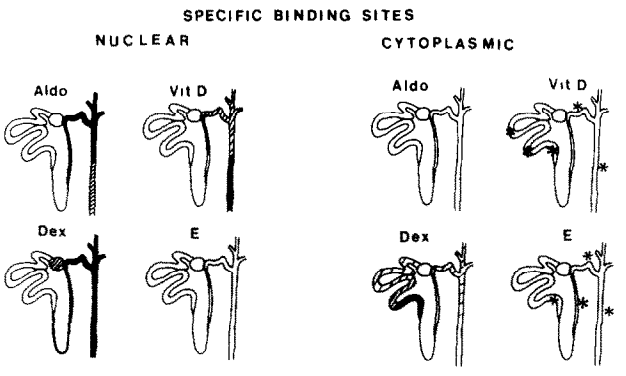

Fig. 3. Nuclear and cytoplasmic binding of steroids along the nephron. Specific nuclear (left) and cytoplasmic (right) binding along the nephron for aldosterone (Aldo), dexamethasone (Dex), 1-25$(OH)_2$ vitamin D_3 (Vit D) and estradiol (E) is schematically represented. Dark zones are those with the maximum specific binding, grey zones with lower or irregular specificity binding, and open zones with no specific binding. Stars indicates zones with no specific binding, but high diffuse non-specific labeling. See text for comments.

also allow us to demonstrate a *de novo* RNA synthesis [5, 6] after *in vivo* administration of DOCA, a potent mineralocorticoid drug. This synthesis occurs all along the collecting tubule. Of interest, the different cell types (principal and intercalated cells) constituting these epithelia exhibit differential responses to mineralocorticoids. This is also the case for aldosterone-induced modulation of basolateral membranes which occurs in principal but not in intercalated cells [16], and for the rate of RNA synthesis after mineralocorticoid administration [17].

Transport studies undoubtedly indicate that aldosterone-induced antinatriuresis [18, 19] depends on the action of this hormone on cortical collecting tubule. All reports give a positive answer about this point. The described effects of the hormone consist of increase in transepithelial potential difference, sodium reabsorption, potassium and proton secretion. These results are obtained either *in vivo*, by tubular micropuncture, or *in vitro*, on perfused microdissected tubules. Among the bulk of experiments in this field, it is noticeable that the more recent ones were performed with very low (0.1–1.0 nM), physiological doses of aldosterone. This allows us to attribute with certainty the observed effect to a mineralocorticoid action induced by aldosterone, in the absence of interference with putative effects due to the binding of aldosterone to glucocorticoid receptors, which occurs at higher doses. A clear dose–response effect has been shown in isolated perfused tubular segments by Schwartz and Burg [20]. It is remarkable that, with very few exceptions, positive results have been obtained only after *in vivo* injection of the hormone, but not when aldosterone was added *in vitro*. This phenomenon is as yet unexplained.

Action of aldosterone in other parts of the nephron is still under discussion. Some authors failed to observe binding [21] or enzyme induction [14] in the distal tubule. However, autoradiographic results clearly show binding in this segment [7, 8], and recent studies using *in vivo* microperfusion demonstrate aldosterone-induced electrolyte transport in distal tubule [22–24].

The observation of an increased sodium concentration in tubular fluid at the end of the loop of Henle after adrenalectomy [25] suggested that aldosterone could modulate sodium reabsorption along this segment. However, the high doses of hormone required to correct the defect and the better efficiency of glucocorticoids [26] argue in favor of a glucocorticoid rather than an aldosterone action. In fact, this point is still under discussion.

No convincing results have been reported on sodium reabsorption modulation by aldosterone in the medullary collecting duct. By contrast, some recent studies evoke [27, 28] an increase of acid excretion in this segment after administration of moderate doses of aldosterone.

Finally, after a very long period of controversy due to the use of high doses, and technical limitations, it seems, in the light of recent studies [29], that aldosterone very probably does not act in the proximal tubule. More detailed data on physiological action of aldosterone along the nephron can be found in recent reviews [30, 31].

Before examining the binding and action of other steroids within the kidney, we would like to consider briefly aldosterone metabolism in this organ. It is certain that kidney cells are able to degrade aldosterone into several metabolites [32]. Consequently, the separation of these metabolites and the question of their quantitative importance as well as of their eventual physiological relevance have been widely explored. We examined recently the presence of metabolites possibly bound to receptors in target (CCT) as compared with non-target (PCT) segments for aldosterone. We found that only some metabolites (more polar than aldosterone) were

bound, but to non-specific sites, in target as well as non-target segments [33]. They were in relatively small amounts (less than 15% of bound aldosterone). In our mind, these observations, together with the very low efficiency of metabolites, as compared with aldosterone, on electrolyte transports, render improbable the existence of a significant physiological action of aldosterone metabolites on kidney function. However, further studies, in particular on isolated tubules, should be required to draw definite conclusions.

DEXAMETHASONE

We determined by autoradiography the specific binding of dexamethasone along the rabbit nephron [10]. Although dexamethasone is not a natural corticosteroid, but a synthetic product, it is considered as a pure glucocorticoid agonist.

Results are illustrated in Fig. 3. A specific nuclear binding of dexamethasone was found in all parts of the tubule, except in the proximal tubule, in its convoluted as well as straight portion. The presence of nuclear binding sites is observed for concentrations as low as 0.15 nM and increases up to 50 nM. The intensity of the specific nuclear labeling is roughly equivalent in all segments beyond the pars recta. These results differ from those obtained with aldosterone in the sense that, with this latter hormone, a clear prevalence of CCT over other segments was present. Anyway, it is clear that all distal parts of the nephron possess both aldosterone and dexamethasone specific nuclear binding sites. From a quantitative point of view, dexamethasone binding sites predominate. An attempt to determine the respective specificity of binding of these two classes of hormones in tubular segments was done by competition studies after incubation with tritiated aldosterone [7]. Results indicated a mixed specificity in all segments which bind aldosterone. An exception was the cortical collecting tubule where a much higher specificity was found for aldosterone than for dexamethasone.

A striking observation, in contrast with aldosterone, was the presence, in the proximal tubule, of dexamethasone specific cytoplasmic binding sites, in the absence of nuclear ones. They are detectable at 3 nM, and reach very high values, equivalent or superior to nuclear ones, at 53 nM. A specific cytoplasmic labeling appears also in DCT and CCT, but only at 53 nM, where it coexists with nuclear labeling. This exclusive proximal cytoplasmic specific labeling increases along this structure, and reaches a maximum at the end of the pars recta. This is illustrated in Fig. 4: the contrast between the nuclear labeling in the thin descending part of the loop of Henle, and the diffuse cytoplasmic labeling in the pars recta is evident. The transition between the two types of labeling occurs within a few microns. Autoradiographs after different times of incubation with tritiated dexamethasone showed that the difference between the two types of labeling is not due to different kinetics of nuclear translocation of hormone–receptor complexes.

The present results on the localization of glucocorticoid binding sites along the tubule roughly agree with the results of Lee *et al.* [34]. These authors measured the binding of corticosterone, a natural steroid in rat and rabbit, with less marked specificity for glucocorticoid sites. Their method did not allow separation of cytoplasmic and nuclear sites. They found a specific binding all along the tubule, with a prevalence in the cortical collecting tubule.

Physiological action of glucocorticoids on the renal function is more confused than for aldosterone. As reported in other chapters, glucocorticoids are responsible for enzyme induction, and particularly ATPase induction. They also play a role in the regulation of renal net glucose release, at the level of the proximal tubules [35, 36]. In the cortical collecting tubule, they seem to modulate basolateral membrane area, as mineralocorticoids do [16]. Their effects on water and solute transports are less well assessed. It is generally believed that they do not act directly on sodium reabsorption, which depends on mineralocorticoid status. Several experiments [18, 19, 37] demonstrate that glucocorticoids largely augment potassium excretion but this result was not found by others [38, 39]. However, several lines of evidence are in favor of an indirect action (via glomerular filtration and tubular flow) rather than a primary effect [23]. Glucocorticoids increase acid excretion by the kidney [40]. This increase could be due to an increase in ammonium, phosphate and titratable acid excretion. This contrasts with aldosterone, which directly lowers urinary pH via proton secretion by tubular cells [40]. For a long time, it was demonstrated that adrenalectomy alters both the ability to excrete a water load, and the concentrating capacity of the kidney [41]. Whether these effects result from an interaction with antidiuretic hormone or not is not assessed with certainty.

The precise site of action of glucocorticoid hormones on water and solute transport along the tubule is still under discussion, and, probably, several sites are involved. Among these putative sites, proximal tubule is very probable. Glucocorticoids were shown to increase phosphate excretion via a proximal effect [42]. In experiments which are now in progress, we show that in Brattleboro rats (with hereditary defect in ADH production), dexamethasone corrects simultaneously the defect in water and phosphate excretion and the increase in glucose Tm (maximal reabsorption) induced by adrenalectomy. The association of these three effects strongly suggests a proximal site of action. The action on phosphate excretion is dose dependent, and aldosterone, even at very high doses, is ineffective. Indeed, since Brattleboro rats lack ADH, the observed glucocor-

ticoid action cannot pass through an interaction with it. If these results are confirmed, we should be in the presence of a rapid (60 min), specific, probably direct action of dexamethasone in a segment with specific cytoplasmic binding sites for this hormone, but lacking nuclear receptors. The question of a non-genomic mechanism of this action, at this site, should thus be raised.

1-25-DIHYDROXYVITAMIN D_3 (1-25$(OH)_2D_3$)

1-25$(OH)_2D_3$ is mainly formed in the kidney from 25$(OH)D_3$. It is considered as the major biologically active metabolite of vitamin D, and binds to specific receptors in several organs [43]. Intestinal mucosa appears to be the main site of binding and action for 1-25$(OH)_2D_3$, so that most studies have been conducted on this tissue.

In the kidney, the site of production of 1-25$(OH)_2D_3$ is the proximal tubule. In a recent work [44], we examined if this hormone also binds in the kidney, either at its site of production, the proximal tubule, or at another tubular site. This work was performed by autoradiography of tritiated 1-25$(OH)_2D_3$ on isolated tubular segments from rats. Before experiments, the endogenous production of 1-25$(OH)_2D_3$ was drastically reduced by injection of disodium ethane-1-hydroxy-1-1-diphosphonate (EHDP), an inhibitor of 1α-hydroxylase activity. Before performing autoradiography, we checked by HPLC of kidney extracts that, after incubation of tissue with tritiated hormone, a large majority of products recovered (about 80–90%) consisted of native 1-25$(OH)_2D_3$.

Results are summarized in Fig. 3. A specific nuclear binding appears in the cortical part of the thick ascending limb of the loop of Henle (CAL) and in the medullary collecting tubule (MCT) at a concentration of 1 nM. This specific nuclear binding remains at a plateau up to 12 nM. A specific nuclear labeling also appears in DCT and CCT, but only at the highest concentration, 12 nM. No specific nuclear labeling was present in other parts of the nephron. In cytoplasm, a non-specific, non-displaceable labeling was present all along the nephron, in segments lacking nuclear specific binding as well as in those with specific nuclear sites. This labeling regularly increases with concentration, and reaches important values, higher than those of specific nuclear binding.

These results have to be compared with those of two other studies, one performed by autoradiography on kidney slices after *in vivo* injection [45, 46], and the other using biochemical methods on pools of isolated tubular segments [46]. Both of them were done in vitamin-D-deficient rats, at variance with our experiment, where EHDP-treated rats were utilized. On the whole, all three studies are consistant in designating the thick ascending limb of the loop of Henle as a target segment for 1-25$(OH)_2D_3$. Concerning other segments, differences exist between the three studies. Stumpf *et al.*[45] found a specific labeling in DCT but not in CCT. This latter segment is also described as non target by Kawashima *et al.*[46]. In contrast, Kawashima *et al.* reported a specific labeling in PCT, a result at variance with ours and that of Stumpf. Medullary collecting tubule was examined only in our study. We have no explanation for these discrepancies. However, two remarks can be made. First, differences probably do not result from the experimental status of the rats, since Stumpf and Kawashima, both using vitamin-D-deficient rats, obtained contradictory results in PCT. Second, a very high level of non-specific binding is present in the PCT of Kawashima, a finding also reported in our study. Consequently, since, by their method, they cannot separate cytoplasmic and nuclear labeling, the precise determination of a weak specific binding in the presence of a high non-specific binding appears difficult.

In the present state of knowledge, it is difficult to establish clear relationships between the localization of specific binding sites for 1-25$(OH)_2D_3$ along the nephron and the eventual role of this hormone in this structure.

Two vitamin-D-dependent calcium-binding proteins (CaBP) have been shown in the rat tubule [47]: one (mol. wt 28,000) in the distal and connecting tubules, and the other (mol. wt 9000–10,000) in all tubular parts beyond the thick ascending limb. Thus, if we consider both CaBP, their localization along the nephron is in good agreement with our results.

With regards to the physiological role of 1-25$(OH)_2D_3$ in regulating ion transport in the kidney, the data presently available in the literature do not provide enough support to establish a clear relationship between binding sites and eventual specific 1-25$(OH)_2D_3$ modulated transport in the kidney.

ESTRADIOL

Although cellular and molecular aspects of sex steroids have been widely studied by endocrinologists, little is known of their eventual specific effects on transport processes by renal epithelia, as well as on the localization of binding sites within the kidney. Specific binding of estradiol has been described in cytosol and nuclei of whole kidney [48]. An antinatriuretic effect of estrogens has also been evoked and attributed to either a direct effect or to interaction with mineralocorticoids [48]. To explore the localization of eventual binding sites along the nephron, we are now looking at autoradiographic labeling by estradiol on isolated tubular segments from immature female rabbits, with very low endogenous hormone.

Preliminary results do not show any specific nuclear or cytoplasmic labeling along the nephron. No clear difference is present between segments,

which all exhibit a significant non-specific diffuse labeling. Such results are not in favor of a specific, well-localized, binding site for this hormone, which could induce a defined primary effect on epithelial transport. Indeed, this does not exclude other possible effects comparable to anabolic actions described for androgens. In fact, the mouse kidney is a widely utilized model for RNA and protein induction by androgens, which are responsible for large sexual kidney dimorphism in this species [49]. This is not so evident, alas, in other species such as rat and rabbit, beloved to renal physiologists....

Before concluding, we would like to give briefly two examples of application of the techniques of localization of binding along the nephron to questions beyond the scope of the strict analysis of the regulation of nephron functions by steroid hormones. One concerns the ontogenic development of kidney functions, and the other is related to the pathophysiology of hypertension.

Ontogenesis of the kidney

Transport functions by the various epithelia progressively appear in the developing kidney, in relation with the cellular maturation of the considered epithelium and the successive steps of the general organization of the organ. One can wonder whether the cellular hormone receptor complex is present before the related function appears. In this view, we examined, by autoradiography, if aldosterone receptors are present in immature developing tubules of kidney of rabbit fetuses. Results show that specific nuclear binding of aldosterone is present in the distal, but not proximal, parts of the nephron 3 days before birth. The localization of this binding thus strictly corresponds to what is observed in the adult. Moreover, the intensity of the labeling, reflecting the number of binding sites, is equivalent in adults and fetuses. At this time, before birth, transport function by distal parts of the nephron is either poorly developed or absent: we found specific binding even in strictly non-filtering nephrons. Thus, these results clearly indicate that the aldosterone receptor system in terminal nephron of fetuses is present, with characteristics similar to those of adults, at a time when electrolyte transport regulated by this system is not functioning.

Pathophysiology of hypertension

A bulk of studies has been developed in a particular strain of rats (Okamoto rats) which spontaneously develop arterial hypertension. Most authors reported that these rats present an alteration of sodium excretion by the kidney, which is susceptible to intervene in the pathogeny of hypertension. This defect seems to be located in distal parts of the nephron where sodium reabsorption is regulated by aldosterone. No alteration of aldosterone secretion or metabolism can be shown in these rats, and aldosterone binding determined by biochemical methods on whole kidney tissue was found to be normal. It should be recalled here that the very specific target segment for aldosterone, CCT, represents only about 5% of the renal mass. We applied our autoradiographic methods of localization of aldosterone binding to spontaneously hypertensive rats [50]. It appears that, in the distal and collecting tubules, the level of specific nuclear binding is clearly increased, in the overall Okamoto strain, as compared with standard Wistar rats. This could be responsible for the elevated sodium reabsorption in these rats. This example shows that differences in aldosterone receptor system, which are masked when experiments are performed on total kidney tissue, can be detected provided that we work on exclusive target segments.

SUMMARY AND CONCLUSION

In this section, we examined the localization of binding and action of four steroids along the nephron: aldosterone, dexamethasone, 1-25-dihydroxyvitamin D_3 and estradiol.

Aldosterone specifically binds to nuclei of the distal and cortical collecting tubule and, to a lesser extent, in the loop of Henle and medullary collecting duct. No binding is present in proximal tubule. Physiological action of aldosterone is located in distal and collecting tubule. It consists mainly of sodium reabsorption and, very probably, of potassium and proton secretion.

Dexamethasone, a pure glucocorticoid synthetic agonist, exhibits a specific nuclear binding all along the distal parts of the nephron, that is beyond the end of the proximal tubule. In the proximal tubule, a cytoplasmic specific binding is apparent in the absence of nuclear binding. Dexamethasone induces several enzymes and favors acid and potassium excretion: in these latter cases, the effect is probably not primary but indirect. It also contributes to regulate water and phosphate excretion.

$1\text{-}25(OH)_2D_3$, formed from $25(OH)D_3$ in the proximal tubule, specifically binds to nuclei of the thick ascending limb of the loop of Henle, to medullary collecting duct and, to a lesser extent, to distal and cortical collecting tubule. This specific nuclear binding is associated with a high non-specific cytoplasmic labeling present all along the nephron. The localization of the specific nuclear binding corresponds to that of the synthesis of CaBP by the tubule. Insufficient information is available to correlate the localization of $1\text{-}25(OH)_2D_3$ binding with a precise solute transport along the tubule.

No specific nuclear binding was found along the nephron for estradiol. We observed only an important non-specific cytoplasmic binding. Estradiol, and other sexual steroids, play probably an important role in the cellular metabolism, in the kidney as in other organs, but our results are not in favor of a precise, localized, intervention of estradiol in tran-

sepithelial transport processes, in particular for sodium, as it has been evoked.

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