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The role of polyester interstitium and aldosterone during structural development of renal tubules in serum-free medium

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Abstract

Little knowledge is available regarding the development of renal stem/progenitor cells into functional parenchyme. To investigate the environmental mechanisms during this maturation process, we elaborated an advanced culture technique to follow renal tubule development. Embryonic stem/progenitor cells derived from neonatal rabbit kidney were placed in a perfusion culture container at the interphase of an artificial polyester interstitium. Tissue culture was carried out in IMDM without serum or protein supplementation and without coating with extracellular matrix proteins. Development of tubules was registered histochemically on cryosections labeled with soybean agglutinin (SBA) and tissue-specific antibodies. The experiments revealed that the development of renal tubules depends exclusively on the administration of aldosterone. The use of 1×10^{-7} M aldosterone for 13 days generated numerous SBA-labeled tubules, while no tubules developed in the absence of the steroid hormone. To obtain further information about the action of the hormone on the cognate receptor, molecular precursors of the aldosterone synthesis pathway were tested. Surprisingly, application of cholesterol, pregnenolone, progesterone, 11-deoxycorticosterone (DOCA) and corticosterone failed to form numerous tubules. Only 11-DOCA and progesterone induced a few tubules, which were barely SBA-labeled. Furthermore, application of aldosterone antagonists such as 1×10^{-4} M spironolactone and 1×10^{-4} M canrenoate completely inhibited the development of tubules. We conclude that specifically aldosterone promotes the development of tubules via the mineralocorticoid receptor whereas its precursors have no effect.

Keywords: Kidney; Tissue engineering; Stem/progenitor cells; Tubules; Artificial interstitium; Aldosterone

1. Introduction

Development of the parenchyme within the kidney is a rather complex process. It starts with the reciprocal interaction between the epithelial cells of the collecting duct ampullae and the surrounding nephrogenic mesenchymal cells [1,2]. Morphological signs of this tissue interaction become visible as the development of an S-shaped body, which develops in close neighborhood of the related collecting duct ampulla. During ongoing development the tubular portions of the nephron arise from the capsule-orientated wing of the S-shaped body, while the medulla-orientated wing forms the glomerulus. Then each tip of a collecting duct ampulla elongates and divides dichotomously. By an unknown signal both epithelia fuse and form the connecting tubule. In consequence, the parenchyme anlage of the kidney arises from two very different kinds of stem/progenitor cells [2–5]. The tubular elements of the proximal tubule, the segments of the loop of Henle and the distal tubule develop from the capsule-orientated wing of the S-shaped body, which in turn are derivates of nephrogenic mesenchymal stem/progenitor cells [6]. In contrast, the collecting duct tubule arises from epithelial stem/progenitor cells initially found in the collecting duct ampullae.

The proceeding tubule development is characterized by a cellular segmentation resulting in specific cells of the proximal tubule, the loop of Henle and the distal tubule [7,8]. Some of the individual portions of the tubule such as the proximal and distal tubule form convolutes, while the segments of the loop of Henle show a straightforward growth from the cortex towards the medulla. Peculiarly, each segment of the nephron tubule is composed of a single

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Table 1
Effects of aldosterone and its molecular precursors on the mineralcorticoid receptor in the adult kidney

Precursors of aldosterone synthesis pathway	Mediated effects	References
Cholesterol	Unknown	None
Pregnenolone	Unknown	None
Progesterone	Antagonist	[47]
11-Deoxycorticosterone	Efficient as aldosterone	[29,48]
Corticosterone	100 times less potent	[30,31]
18-hydroxycorticosterone	Unknown	[49]
Aldosterone	Maximal effect	[20,44]

cell type. In contrast, the connecting and the collecting duct tubule exhibit a heterogenous cell population consisting of principal cells (P) and various types of intercalated cells (IC) [9]. Up to date it is unclear, which molecular processes are involved in the formation of nephron and collecting duct tubule segmentation and in their three-dimensional extension during organ growth.

Our interest is focused on the cell biological mechanisms involved in the three-dimensional development of a model tubule derived from renal stem/progenitor cells. Such a process is pleiotropic and starts with few stem/progenitor cells, produces numerous maturing cells, leads to the formation of a three-dimensionally structured tubule, steers the elongation of the individual segment and gradually generates a polarized epithelium [10–13]. Finally, a functional tubule is formed with a defined length, a distinct polarization and a constant inner and outer diameter [14]. Furthermore, morphogenic information must be present promoting either the development of a straightforward-orientated segment or a curved growth within a convoluted tubule. Reviewing critically these basic events of tubulogenesis within the developing kidney one needs to admit that the mechanisms driving most of the mentioned processes can not be explained yet. However, regarding the dramatic clinical course of renal failures and the challenges in regenerative medicine covering this field, exact knowledge of tubule development will be of special importance in the future to find the optimal strategy to treat patients with stem or progenitor cells [15–19].

To investigate the environmental needs of maturing tubules, the availability of a powerful culture system is of fundamental importance. One of the necessities is to experimentally influence embryonic tissue so that it generates tubules which can be maintained in culture over prolonged periods of time. In a previous paper, we showed the feasibility of culturing renal tubules derived from embryonic tissue at the interphase of an artificial interstitium made of polyester fleece within a perfusion culture container [20,21]. Applying this innovative in vitro model, the coating of embryonic renal tissue with extracellular matrix proteins is not necessary. Furthermore, the experiments can be performed in a chemically defined medium without any serum or protein supplementation. Using this innovative approach we found that the generation of renal tubules depends on the administration of aldosterone. The mineralocorticoid acts in a concentration-dependent manner with an optimum concentration range of 1×10^{-7}–1×10^{-6} M. The developmental process requires an unexpected long period of 8 days until the first signs of polarized tubules become visible.

It is a surprising finding that aldosterone exhibits tubulogenic activity in embryonic renal tissue [20]. In the adult kidney the hormone does not show any tubulogenic activity but rather regulates physiological functions by genomic and non-genomic effects [22–25]. Besides aldosterone also some of its molecular precursors have an affinity to the mineralocorticoid receptor and influence physiological functions (Table 1). In this context it is unknown, if precursors of the aldosterone synthesis pathway also show effects on development of renal stem/progenitor cells. Consequently, using embryonic renal tissue we investigated the tubulogenic activity of aldosterone in comparison with its molecular precursors. We found that specifically aldosterone exhibits a tubulogenic action. Inhibition of aldosterone-induced tubule development by spironolactone and canrenoate suggests that aldosterone acts via the mineralocorticoid receptor. In contrast, the glucocorticoid dexamethasone was not able to mimic the tubulogenic action obtained by aldosterone.

2. Materials and methods

2.1. Isolation of embryonic explants containing renal stem/progenitor cells

One-day-old New Zealand rabbits were anesthetized with ether and killed by cervical dislocation. Both kidneys were removed immediately. Each kidney was dissected in two parts. By stripping off the capsula fibrosa with fine forceps an embryonic tissue layer is harvested containing numerous collecting duct ampullae, S-shaped bodies and nephrogenic mesenchyme [26].

2.2. Perfusion culture of renal tubules at the interphase of an artificial interstitium

For a long-term perfusion culture the isolated tissue was mounted in a tissue holder with 14 mm outer diameter, which is placed in a culture container with horizontal flow characteristics (Minucells and Minutissue, Bad Abbach, Germany; www.minucells.de) as earlier described [20]. To minimize the dead fluid volume within the perfusion culture container the freshly isolated embryonic renal tissue was cultured between two layers of highly porous biocompatible polyester fleece (Walraf, Grevenbroich,

Germany) used as an artificial interstitium. Thus, the embryonic tissue and the polyester material were in close contact. Always fresh serum-free IMDM (I, Iscove's Modified Dulbecco's Medium including Phenolred, GIBCO/Invitrogen, Karlsruhe, Germany) was provided. An antibiotic-antimycotic solution (1%, GIBCO) was added to all culture media. Furthermore, up to 50 mmol/l HEPES (H, GIBCO) was added to the medium to maintain a constant pH of 7.4 under atmospheric air containing 0.3% CO_2. Culture was performed 13 days. The medium was continuously perfused at a rate of 1 ml/h with an IPC N8 peristaltic pump (Ismatec, Wertheim, Germany). To maintain a constant temperature of 37 °C, the perfusion culture container was placed on a thermoplate (Medax, Kiel, Germany) and covered by a transparent lid (Fig. 1). Aldosterone (A) was commercially obtained from Fluka (Taufkirchen, Germany), while cholesterol, pregnenolone, progesterone, 11-deoxycorticosterone (DOCA) and corticosterone were ordered from Sigma (Taufkirchen, Germany). Only one company offers 18-hydroxycorticosterone. This substance was ordered from Steraloids (Newport, USA), but was not available after months. Consequently this metabolite could not be tested. All other hormones were applied in a concentration of 1×10^{-7} M in chemically defined IMDM. Antagonists such as spironolactone and canrenoate were obtained from Sigma.

2.3. Lectin- and antibody-labeling

Cryosections of 20 μm thickness were fixed in ice-cold ethanol. After washing with phosphate-buffered saline (PBS) the sections were blocked with PBS containing 1% bovine serum albumin (BSA) and 10% horse serum for 30 min. For lectin-labeling the specimens were exposed to fluorescein-isothiocyanate (FITC)-conjugated Soybean Agglutinin (SBA, Vector Laboratories, Burlingame, USA) diluted 1:2000 in blocking solution for 45 min as described earlier [27]. For antibody labeling mab anti-occludin (Zymed, San Francisco, USA) and mab anti-laminin γ1 (kindly provided by Dr. L. Sorokin, Lund, Sweden) were applied as primary antibodies for 1 h in blocking solution after washing in PBS. The specimens were incubated for 45 min with donkey-anti-mouse-IgG-fluorescein-isothiocyanate (FITC) respectively goat-anti-rat-IgG-rhodamine (Jackson Immunoresearch Laboratories, West Grove, USA) diluted 1:50 in PBS containing 1% BSA. Following several washes in PBS, the sections were embedded with Slow Fade Light Antifade Kit (Molecular Probes, Eugene, USA) and then analyzed using an Axioskop 2 plus microscope (Zeiss, Oberkochen, Germany). Fluorescence images were made by a digital camera with a standard exposure time of 1.3 s and thereafter processed with Corel DRAW (Corel Corporation, Ottawa, Canada).

2.4. Histological examination and scoring

2.4.1. Definition of generated tubules

Developed tubules were described in longitudinal- or cross-sectioned view as structures showing polarized cells inside SBA-labeled elements. In this case a lumen was visible and a basal lamina bordered the smooth outer surface of a tubule. No filopodia and no overgrowth of cells on the polyester fibers were observed. According to the histochemical profile of SBA-label we used a specific score for evaluation. Presence of tubules, intensity, lumen, basal lamina, amount and length were registered. Each criterion was scored with one point, resulting in a maximum of 6 points.

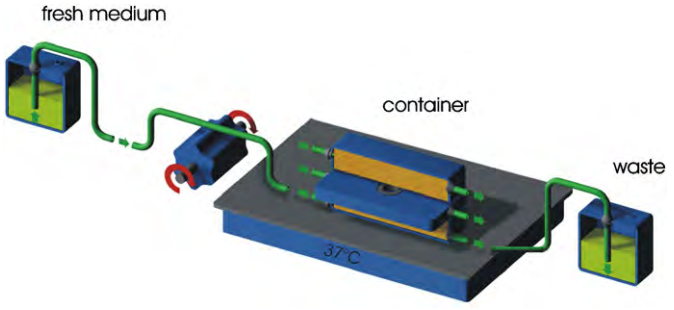

Fig. 1. Schematic illustration of perfusion culture. Fresh culture medium is constantly transported by a peristaltic pump (1 ml/h) into a perfusion culture container, which is placed on a thermoplate at 37 °C. The culture medium is collected in a waste bottle and is not re-circulated.

2.4.2. Definition of cell islets and clusters

An islet was defined as a group of few aggregated SBA-labeled cells. Frequently the cells showed an overgrowth along the polyester fibers. A cluster was defined as an aggregate of many SBA-labeled cells. The diameter of a cluster varied between 30 and 150 μm, it did not show polarization. In some cases traces of a discontinuous basal lamina were visible.

2.5. Amount of cultured constructs

A total of 250 tissue constructs was generated for the present study. All of the experiments were performed at least in triplicates. The data given in the text are the mean of at least three independent experiments. All experiments are in accordance of the animal ethics committee, University of Regensburg, Regensburg, Germany.

3. Results

The aim of the first set of experiments was to investigate the influence of aldosterone and its molecular precursors on tubulogenesis. Embryonic renal tissue was placed in a perfusion culture container filled with a polyester fleece as artificial interstitium (Fig. 2a). Harvested cultures showed that a renal microtissue with a diameter of 5 mm had developed at the interface of the polyester artificial interstitium (Fig. 2b and c).

3.1. Screening for developed tubules

For quick detection the development of tubules was screened with SBA-label. The lectin recognized terminal *N*-acetylgalactosamine (GalNAcα1) residues on glycoproteins [28]. Application of the described titer of fluorescent SBA labeled only cells in matured collecting duct of the kidney (Fig. 3). The isolated tissue layer containing renal stem/progenitor cells corresponded to the zone close to the organ capsule and did not show reaction with SBA [26]. Thus, only matured tubules were recognized by SBA-label.

3.2. Tubulogenic action of aldosterone

In a first series of control experiments we investigated the development of isolated embryonic explants in serum-free standard culture medium (IH) consisting of IMDM (I) with HEPES (H) for 13 days. Cryostat sections of three different specimens showed a disintegration of tissue (Fig. 4, IH). Only thin rows of cells and cell islets were developed. In none of the samples we could observe SBA-positive polarized tubules. The SBA-label demonstrated that some cells grew constantly in close contact on the surface of the polyester fibers.

In contrast, in a second series of experiments after the administration of aldosterone (A) to the standard medium

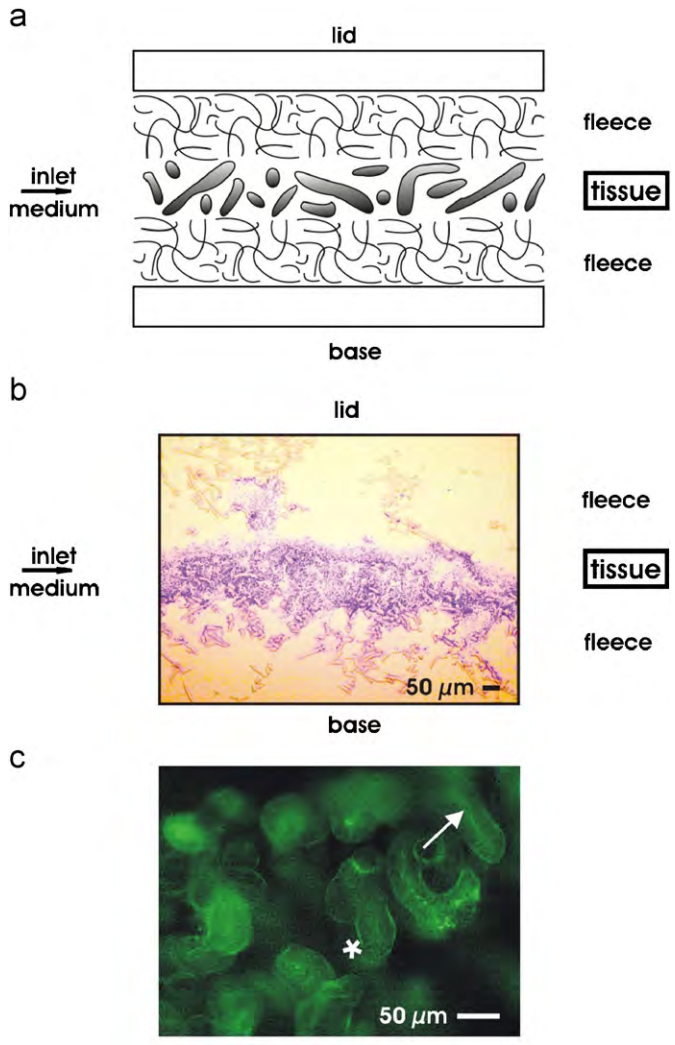

Fig. 2. Generation of renal parenchyme at the interphase of an artificial interstitium. Schematic view into a perfusion culture container (a). The space between the lid and base is filled with an artificial interstitium made of a polyester fleece, where the tissue develops. The microscopic view of a cryosection stained with Toluidine blue reveals generated renal parenchyme (b). The section shows that the tissue is not growing within the fiber space but at the interphase lining to the upper and lower fleece. The surface view demonstrates numerous tubules grown in the artificial interstitium. The asterisk indicates the basal lamina, while the arrow marks a lumen (c).

(IH) there was a complete change in the developmental pattern (Fig. 4, IH-A). In whole-mount specimens we found in a microscopic area of $620 \times 930\,\mu m^2$ between 41 and 74 individual SBA-labeled tubules. The developed tubules were further analyzed in cross- and longitudinal-sectioned view. As revealed by laminin γ1-immuno-label polarized cells formed a basal lamina (Fig. 5a–f). The outer surface of tubules appeared smooth. Immuno-label with occludin further showed that the majority of tubules had multiple and intensively labeled strands within the tight junctions (Fig. 5a′–d′). However, some of the tubules only showed to a minor degree occludin-labeling (Fig. 5e′), while few of them lacked this marker (Fig. 5f′).

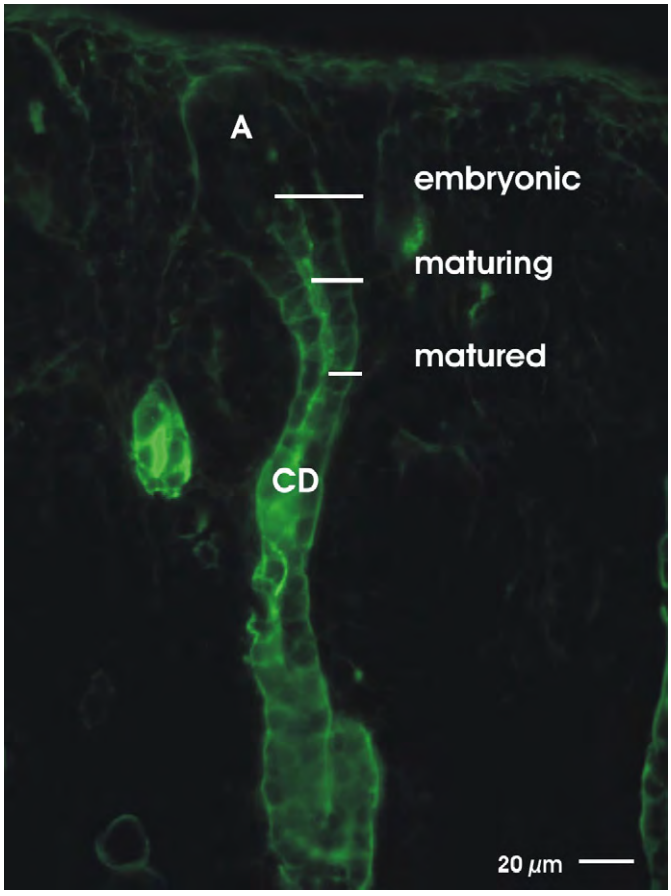

Fig. 3. SBA-label of the embryonic cortex of neonatal rabbit kidney. Only the maturing neck and matured shaft of a collecting duct (CD) ampulla shows a cellular reaction with SBA. In contrast, the tip of the ampulla (A) and the surrounding embryonic tissue are negative for SBA. This embryonic zone was isolated and used for culture. It is completely negative for the SBA-label.

3.3. *Aldosterone versus its molecular precursors*

Of special interest was the question, if exclusively aldosterone or also its precursors show a comparable tubulogenic action. The synthesis of aldosterone starts from cholesterol, which is metabolized over pregnenolone to progesterone, 11-DOCA, corticosterone and 18-hydroxycorticosterone [22]. Physiological experiments with adult kidneys showed that 11-DOCA is as effective as aldosterone on the mineralocorticoid receptor [29], while corticosterone is 100 times less potent [30,31] (Table 1). In this context, it is unknown, if only aldosterone or also its precursors show a similar morphogenic action in embryonic renal tissue (Figs. 6 and 9).

We found that the application of cholesterol (Fig. 6a,a′,a″) and pregnenolone (Fig. 6b,b′,b″) did not result in the formation of any SBA-positive tubules (0 point). Treatment with progesterone (Fig. 6c,c′,c″) led to the development of few tubules, however, without intensive SBA-label (1 point). When 11-DOCA (Fig. 6d,d′,d″) was used, few tubules with a faint SBA-label could be detected (1 point). In contrast, administration of corticosterone (Fig. 6e,e′,e″) did not reveal

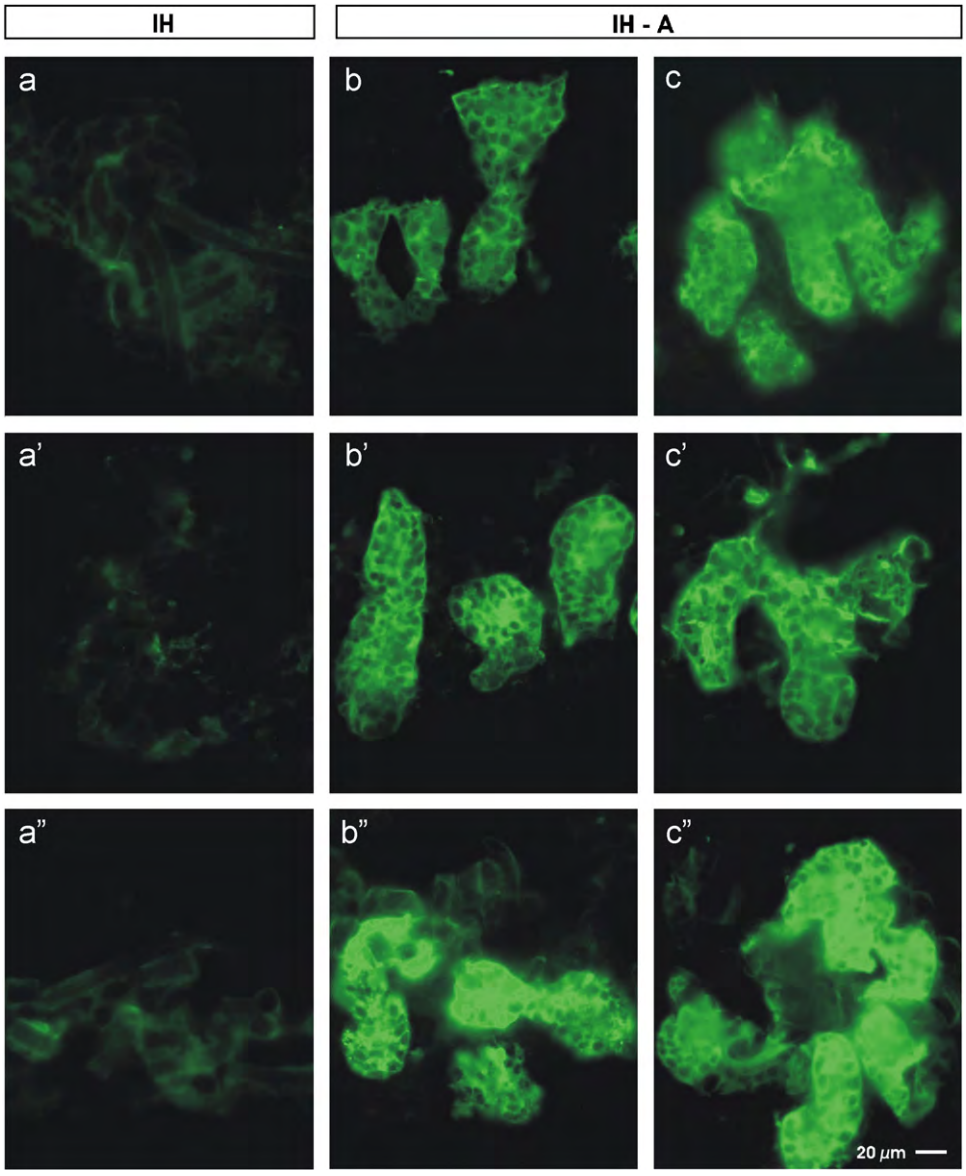

Fig. 4. Tubulogenic action of aldosterone in renal tissue. For control, embryonic renal tissue was cultured for 13 days in serum-free IMDM containing HEPES (IH). Three different examples show that only few SBA-labeled cells develop when hormone supplement is omitted (a–a″). In contrast, when 1×10^{-7} M aldosterone is added to the culture medium (IH-A) numerous and intensively SBA-labeled tubules are detected by SBA-label (b–b″ and c–c″).

any development of tubules. Instead, numerous unstructured SBA-labeled cells in close contact to polyester fibers were observed (0 point). In a control experiment, application of aldosterone (Fig. 6g,g′,g″) resulted in numerous SBA-positive tubules with a distinct lumen and a clearly recognizable basal lamina (6 points). Data for 18-hydroxycorticosterone are missing, since to date this substance was not commercially available (Fig. 6f,f′,f″).

3.4. Antagonists and aldosterone

A further set of experiments was performed to interfere with the morphogenic action of aldosterone (Fig. 7a). The effect of aldosterone (1×10^{-7} M) could not be abolished applying 1×10^{-7} and 1×10^{-6} M spironolactone (not shown). However, application of 1×10^{-5} M spironolactone (not shown) decreased the number of SBA-labeled tubules. No SBA-labeled tubules were present in the presence of spironolactone at a concentration of 1×10^{-4} M (Fig. 7b). Similar results were obtained for the application of canrenoate. The use of 1×10^{-7} and 1×10^{-6} M canrenoate did not affect the development of SBA-labeled tubules. However, the application of 1×10^{-5} M (not shown) canrenoate showed a decrease, while the use of 1×10^{-4} M resulted in a lack of SBA-labeled tubules (Fig. 7c). These results indicated that the tubulogenic action of aldosterone is mediated via the mineralocorticiod receptor.

3.5. Glucocorticoid versus aldosterone

Finally, we investigated whether the tubulogenic action of aldosterone is a specific mineralocorticoid-mediated

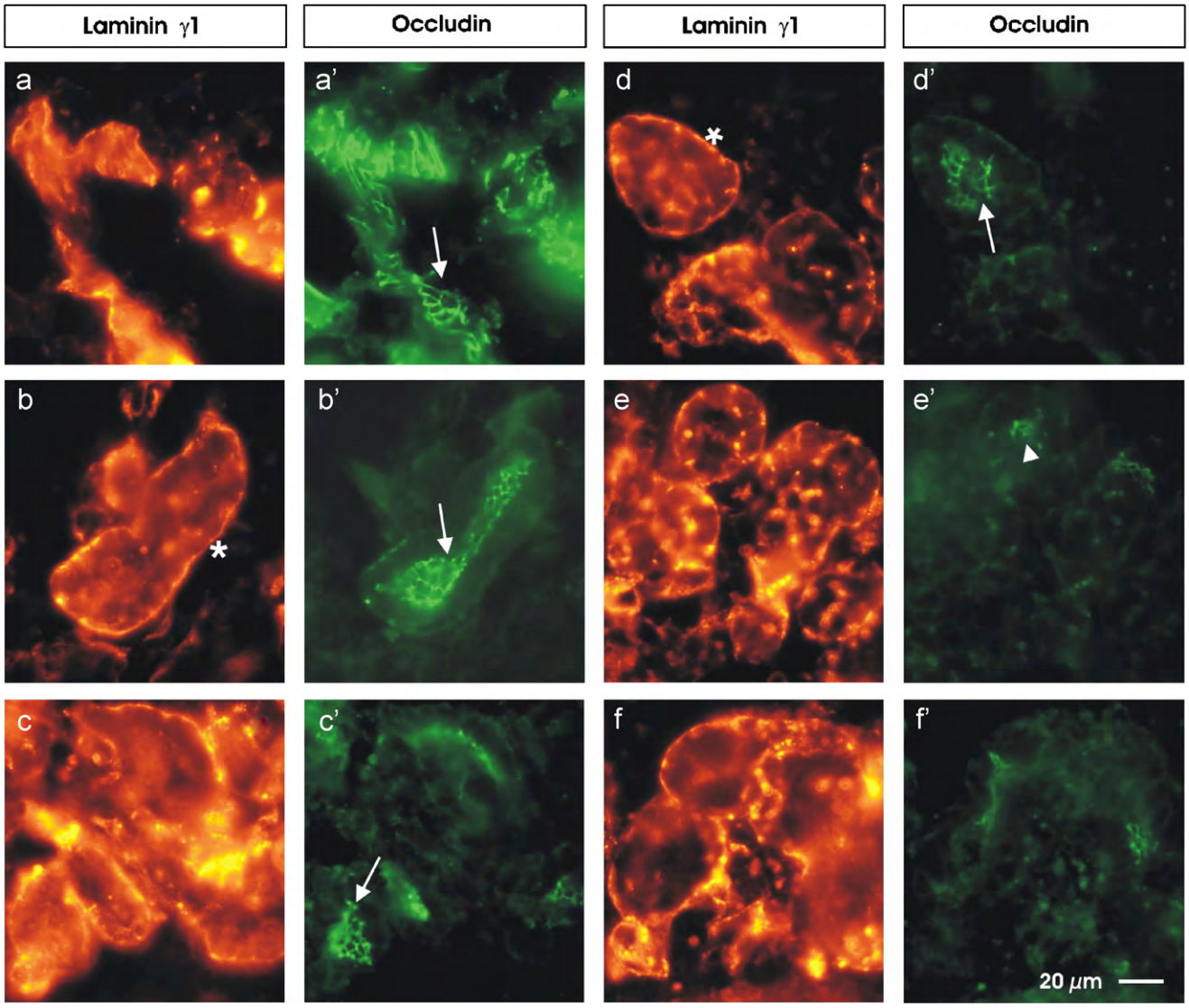

Fig. 5. Immuno-histochemical features of generated renal tubules cultured in aldosterone containing medium (IH-A). Label for laminin γ1 (a–f) shows that tubules develop a continuous basal lamina (b,d, asterisk). Most of the tubules show an intensive label for occludin (a′–d′, arrow), while few reveal minor (e′, arrowhead) or even no occluding-labeling (f′).

effect or may also be triggered by the glucocorticoid receptor. Consequently, we performed a last set of experiments using dexamethasone instead of aldosterone. While the application of aldosterone (1×10^{-7} M) resulted in the development of numerous SBA-labeled tubules (Fig. 8a), the administration of dexamethasone (1×10^{-7} M) produced huge clusters of non-polarized cells (Fig. 8b). In none of the cases dexamethasone was able to generate tubules similar to those observed in the series using aldosterone.

4. Discussion

4.1. Advanced culture of embryonic renal tissue

Due to the limited size of embryonic kidney tissue in mouse [32] or rat [33] specimens, we selected the neonatal rabbit as cell biological model to investigate tubule formation. In contrast to other experiments the use of neonatal rabbit kidney exhibited several advantages [11,34]. The outer cortex of the neonatal rabbit kidney contained numerous stem cell niches in their original extracellular environment. This layer of embryonic tissue was easily accessible for isolation and could be harvested in sufficient amounts for perfusion culture or cell biological analysis. A further advantage was that the embryonic renal tissue can be isolated without enzymatic disintegration so that it remained in its original extracellular environment. For culture, the explants were placed between two layers of a polyester fleece within a perfusion culture container. Coating of the tissue or of the polyester fleece by extracellular matrix compounds was not necessary [35,36]. Application of serum to the culture medium was omitted to avoid any effect of hormones or growth factors contained in the serum.

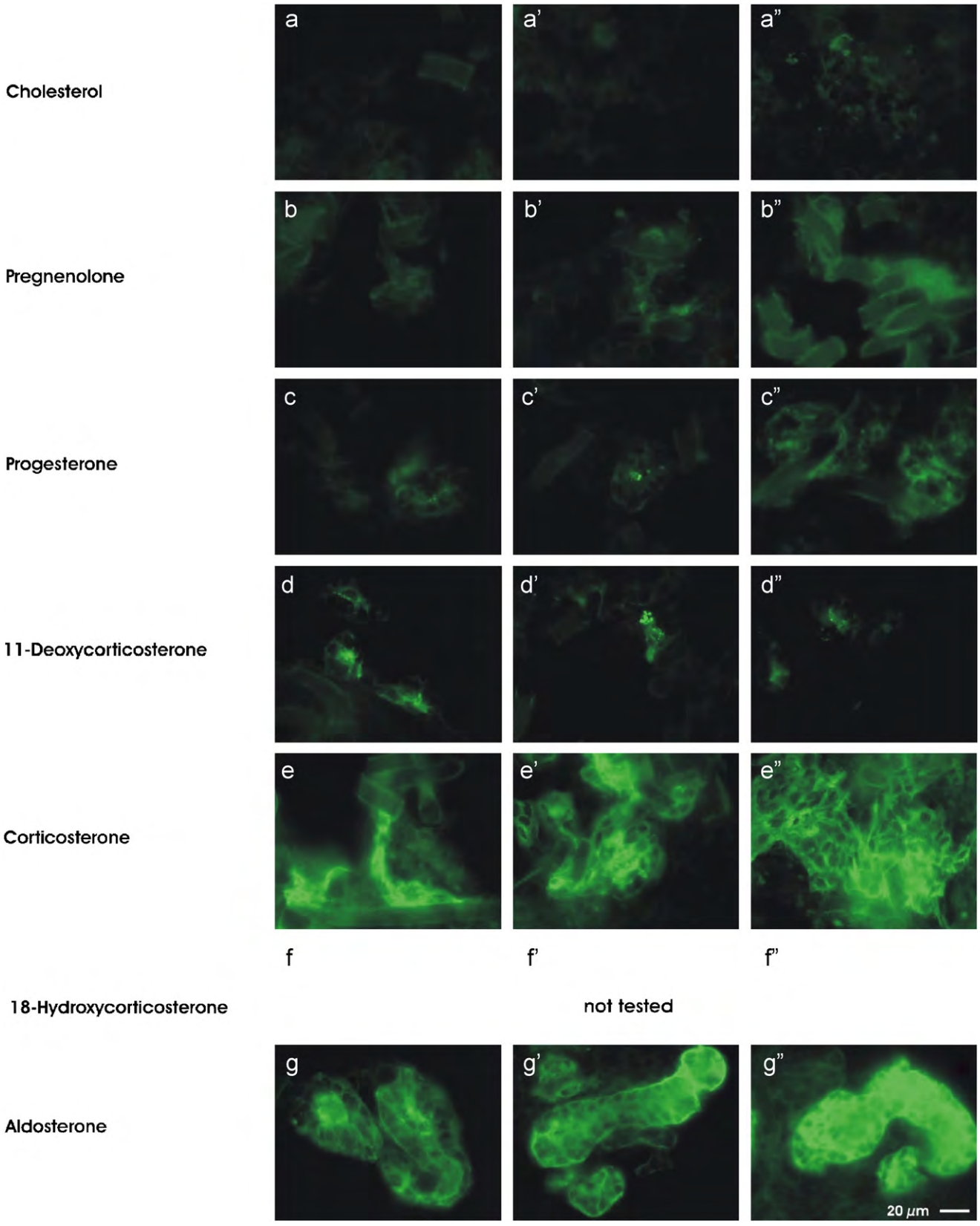

Fig. 6. Culture of embryonic renal tissue for 13 days in chemically defined IMDM containing HEPES (IH) and molecular precursors of the aldosterone synthesis pathway. Administration of cholesterol (Fig. 6a,a′,a″) or pregnenolone (Fig. 6b,b′b″) does not reveal a morphogenic action. Progesterone (Fig. 6c,c′,c″) and 11-deoxycorticosterone (Fig. 6d,d′,d″) show very little action on tubule formation. Corticosterone (Fig. 6e,e′,e″) leads to an overgrowth of cells on polyester fibers but no tubule formation. The effect of 18-hydroxycorticosterone (Fig. 6f,f′,f″) is not tested. For control, aldosterone (Fig. 6g,g′,g″) promotes an intensive formation of renal tubules.

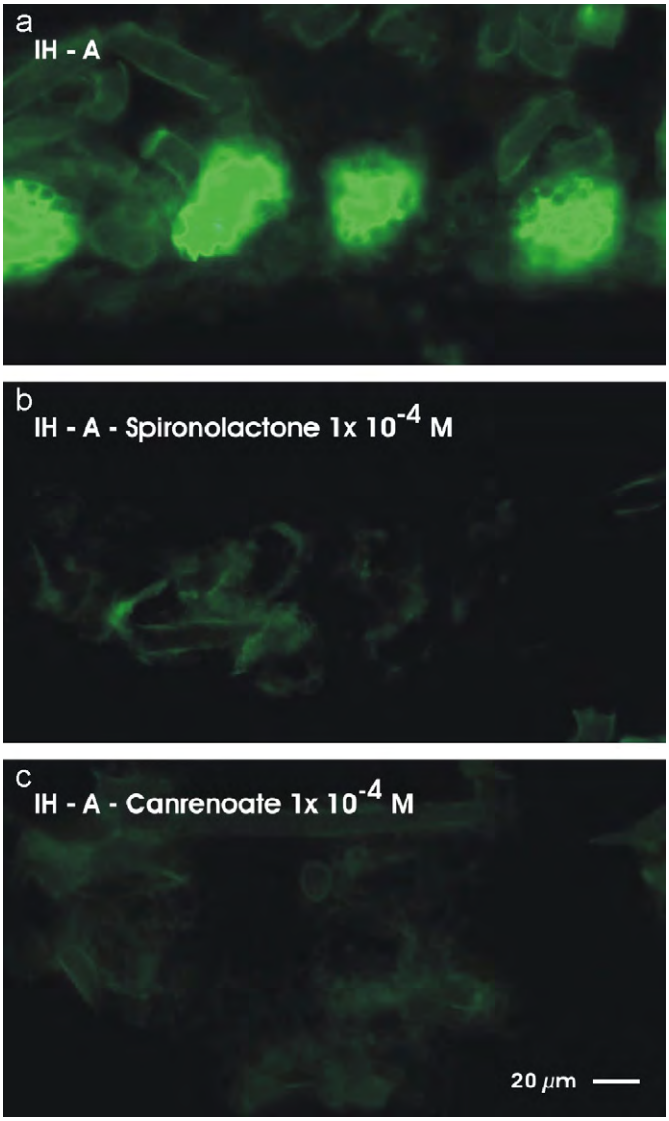

Fig. 7. The tubulogenic action of aldosterone can be inhibited on the receptor level. For control, aldosterone generates numerous SBA-labeled tubules (a). Application of 1×10^{-4} M spironolactone (b) and 1×10^{-4} M canrenoate (c) completely inhibits the aldosterone-dependent development of tubules on the mineralocorticoid receptor.

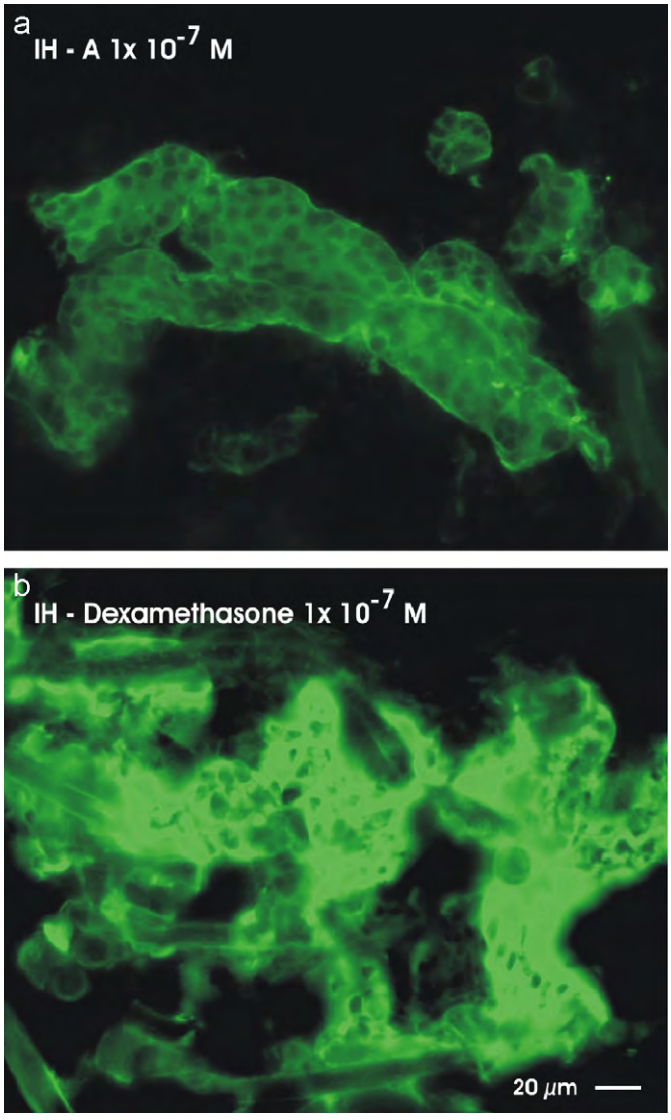

Fig. 8. Dexamethasone does not mimic the tubulogenic action of aldosterone. While aldosterone generates numerous SBA-label tubules in embryonic renal tissue (a), dexamethasone produces numerous extended cell clusters closely attached to polyester fibers (b).

4.2. *Tubulogenic effect of aldosterone*

Commonly used growth factor supplements in renal tissue culture were serum, epidermal growth factor (EGF [19], insulin [37], transferrin [38], selenium [39], retinoic acid [40] (RA), bovine pituitary extract [41] (BPT) or cholecalciferol [42]. However, all of these supplements used individually or in combination failed in our experiments to generate renal tubules [20]. In contrast, the application of the mineralocorticoid aldosterone demonstrated a tubulogenic action leading to the formation of numerous tubules derived from renal stem cells (Fig. 4b–b″, c–c″). Histochemistry further revealed the development of segments of renal collecting duct-derived tubule. This could be recognized by SBA-label (Fig. 3), but also by cytokeratine 19 and TROMA-1 occurrence as shown earlier [20,21]. Immuno-label with mab anti-laminin γ1 (Fig. 5a–f) in the present experiments demonstrated the primary development of a basal lamina. Luminal polarization was recognized by mab anti-occludin (Fig. 5a′–f′), which illustrated the primary arise of tight junctions. The majority of SBA- and laminin γ1-positive tubules (Fig. 5a–f) exhibited intensive occludin-label (Fig. 5a′–d′), while few of them revealed a mediocre staining (Fig. 5e′) and some of them were occludin-negative (Fig. 5f′). It remained unclear, if a maturation gradient within the tissue or if a too short culture period accounted for this effect. In any case, the tubular features supported the assumption that an apico-basal polarization had developed in the cultured tubules after the application of aldosterone.

4.3. Unspecific versus specific steroidal action

One may argue that the effect of aldosterone on tubulogenesis might be related to an unspecific action of a steroidal hormone on the mineralocorticoid receptor in embryonic tissue [30,43]. However, application of dexamethasone produced numerous huge cell clusters (Fig. 8b) instead of tubules (Fig. 8a) [44]. The use of other steroidal hormones such as testosterone [45] or estrogen [46] did not result in the formation of tubules in our experiments (data not shown). These results further suggested a specific steroidal action of aldosterone on a cognate receptor [22–24].

4.4. Involvement of the mineralocorticoid receptor

More insight for a specific action of aldosterone on the mineralocorticoid receptor was obtained by investigating the effects of its precursors on tubular development (Figs. 6 and 9). For example, we did not expect cholesterol and pregnenolone to have tubulogenic activity [25] (Table 1), since no related data in the literature for these compounds could be found. Also, a tubulogenic action of progesterone could not be expected, since it acts in the adult kidney as an antagonist [47]. In contrast, 11-DOCA was described in physiological experiments to be as potent as aldosterone [29,48]. Corticosterone showed agonistic characteristics, but it is 100 times less potent than aldosterone [30,31]. At least the latter precursors of the aldosterone synthesis pathway were expected to exhibit tubulogenic activity. In contrast, no data are available regarding a tubulogenic effect of 18-hydroxycorticosterone [49].

In the present culture experiments, precursors of the mineralocorticoid synthesis pathway such as cholesterol (Fig. 6a,a′,a″), pregnenolone (Fig. 6b,b′,b″), progesterone (Fig. 6c,c′,c″), 11-DOCA (Fig. 6d,d′,d″) or corticosterone (Fig. 6e,e′,e″) did not promote the generation of tubules. 18-hydroxycorticosterone (Fig. 7f,f′,f″) could not be tested, since the substance was commercially not available. Thus, our data suggested a specific action of aldosterone on the mineralocorticoid receptor. None of the precursors showed an effect comparable to that obtained after aldosterone treatment (Fig. 6g,g′,g″). These data further revealed that the tubulogenic action of aldosterone is specific for this hormone. However, the response of the receptor in embryonic renal tissue seemed to be different from the known mineralocorticoid effect in the adult kidney, since 11-DOCA did not show any tubulogenic effects (Fig. 6d,d′,d″). Thus, we demonstrated for the first time that the precursors of aldosterone synthesis pathway do not have a comparable effect on the generation of tubules as it was observed for aldosterone.

Finally, the tubulogenic action of aldosterone (Fig. 7a) could be completely abolished by spironolactone (Fig. 7b) and canrenoate (Fig. 7c). These results further supported the assumption that aldosterone triggers its tubulogenic action via the classical mineralocorticoid receptor and presumably a genomic pathway in embryonic renal tissue.

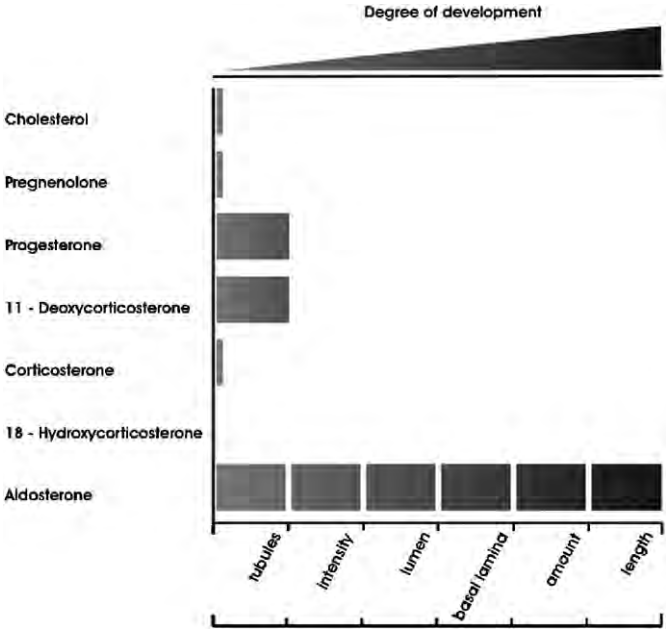

Fig. 9. Histogram of the tubulogenic effect of aldosterone and its molecular precursors on renal tubule development. It depicts that only aldosterone but not its precursors lead to the development of SBA-labeled tubules. Given data are means of at least three independent experiments.

5. Conclusions

Previous data showed that renal stem/progenitor cells develop after administration of aldosterone at the interphase of an artificial polyester interstitium structured tubules. The present experiments now illuminated that application of molecular precursors of aldosterone synthesis such as cholesterol, pregnenolone, progesterone, 11-deoxycorticosterone (DOCA) and corticosterone failed to form tubules. Only 11-DOCA and progesterone induced to a low degree tubules. In addition, spironolactone and canrenoate in the presence of aldosterone completely antagonized the development of tubules. Thus, it is a new finding that specifically aldosterone and not the molecular precursors promoted via the mineralocorticoid receptor (MR) the generation of renal tubules.

Acknowledgments

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