INTRODUCTION

The entry and the transport of sodium in tight epithelia, namely the renal collecting duct and the distal colon, is primarily mediated by an amiloride-sensitive sodium channel. This channel has been widely studied, first because it controls the entry and the reabsorption of sodium in a rate-dependent manner, and second because it participates in the control of cell volume (Benos et al., 1987; Palmer, 1992). It is highly selective for sodium, has a low conductance (4-5 pS), and its activity is regulated by the mineralocorticoid hormone aldosterone (Palmer, 1992).

Several proteins have been purified from bovine renal papilla, which need to be at least partly associated to constitute a functional channel (Benos et al., 1987), while in rat, three subunits, α, β and γ, of the epithelial sodium channel have been recently cloned (Canessa et al., 1993, 1994; Lingueglia et al., 1993). The injection of the α subunit mRNA into Xenopus oocytes generated a channel whose activity was low. Expression of either β or γ subunits alone or in combination did not lead to any measurable current. Coexpression of α and β or α and γ resulted in a small amiloride-sensitive Na⁺ current. However, the coinjection of the three subunit mRNAs generated a channel which was highly specific for sodium, had a low conductance, and whose activity was inhibited by amiloride (Canessa et al., 1994). To date, there is no clear relationship between the cloned subunits and each of the proteins purified from the bovine papilla (Benos et al., 1987).

The proteins of each of the subunits are 30-40% homologous, suggesting that they may derive from a common ancestor. Interestingly, the genes coding for these proteins also have significant homologies with mec-4, mec-10 and deg-1, a family of Caenorhabditis elegans genes whose translation products are involved in sensory touch transduction. Mutations in these genes result in a touch-insensitive phenotype, and neuronal degeneration (Driscoll and Chalfie, 1991). Neurons in the dominant mutants exhibit a cytoplasmic swelling which is followed by a vacuolization and then cell death. This process is attributed to an abnormal control of the cell volume (Driscoll and Chalfie, 1991). Thus the amiloride-sensitive sodium channel of the rat (rENac) may be part of a family of genes that code for cation channels that regulate cell volume.

SUMMARY

Three subunits (α, β, γ) of the amiloride-sensitive epithelial sodium channel have been recently characterized. The channel subunits have significant homologies with the Caenorhabditis elegans mec-4, mec-10 and deg-1 genes, which are involved in control of cell volume and mecano-transduction. These subunits are coexpressed at equivalent levels in the renal collecting duct and the distal colon epithelium which are high resistance sodium transporting epithelia. We have investigated whether these subunits were expressed, at the mRNA level, in transporting as well as non transporting epithelial cells of rat skin. In full-thickness abdominal skin only α and γ subunit mRNAs were detected, while all three subunit mRNAs were present in sole skin, as demonstrated by RNase-protection assay. Furthermore, the level of expression of each subunit varied with the epithelial cell type as demonstrated by in situ hybridization: epidermal and follicular keratinocytes express mostly α and γ subunits (while β was low); a prevalence of β and γ was observed in sweat glands. Thus, it appeared that two out of the three subunit mRNAs predominated in each epithelial structure. In addition, mRNAs of the α, β and γ subunits of the amiloride-sensitive sodium channel were expressed at a higher level in large suprabasal epidermal keratinocytes (which undergo terminal differentiation) than in small proliferative basal keratinocytes.

Key words: In situ hybridization, RNase protection, Sweat gland, Hair follicle, Epidermis

INTRODUCTION

The entry and the transport of sodium in tight epithelia, namely the renal collecting duct and the distal colon, is primarily mediated by an amiloride-sensitive sodium channel. This channel has been widely studied, first because it controls the entry and the reabsorption of sodium in a rate-dependent manner, and second because it participates in the control of cell volume (Benos et al., 1987; Palmer, 1992). It is highly selective for sodium, has a low conductance (4-5 pS), and its activity is regulated by the mineralocorticoid hormone aldosterone (Palmer, 1992).

Several proteins have been purified from bovine renal papilla, which need to be at least partly associated to constitute a functional channel (Benos et al., 1987), while in rat, three subunits, α, β and γ, of the epithelial sodium channel have been recently cloned (Canessa et al., 1993, 1994; Lingueglia et al., 1993). The injection of the α subunit mRNA into Xenopus oocytes generated a channel whose activity was low. Expression of either β or γ subunits alone or in combination did not lead to any measurable current. Coexpression of α and β or α and γ resulted in a small amiloride-sensitive Na⁺ current. However, the coinjection of the three subunit mRNAs generated a channel which was highly specific for sodium, had a low conductance, and whose activity was inhibited by amiloride (Canessa et al., 1994). To date, there is no clear relationship between the cloned subunits and each of the proteins purified from the bovine papilla (Benos et al., 1987).

The proteins of each of the subunits are 30-40% homologous, suggesting that they may derive from a common ancestor. Interestingly, the genes coding for these proteins also have significant homologies with mec-4, deg-1, and mec-10, a family of Caenorhabditis elegans genes whose translation products are involved in sensory touch transduction. Mutations in these genes result in a touch-insensitive phenotype, and neuronal degeneration (Driscoll and Chalfie, 1991). Neurons in the dominant mutants exhibit a cytoplasmic swelling which is followed by a vacuolization and then cell death. This process is attributed to an abnormal control of the cell volume (Driscoll and Chalfie, 1991). Thus the amiloride-sensitive sodium channel of the rat (rENac) may be part of a family of genes that code for cation channels that regulate cell volume.
Each of the subunits of the rENac is expressed at comparable levels in tight epithelia which are involved in sodium reabsorption, in the distal and collecting renal tubules, the distal colonic epithelium and the striated ducts of the salivary glands (Duc et al., 1994). In these epithelia, the activity of the amiloride-sensitive sodium channel is regulated by the mineralocorticoid hormone aldosterone. For instance, the mean channel opening time is increased in presence of aldosterone in the rat cortical collecting duct (Palmer, 1992) which expresses the mineralocorticoid receptor (Farman and Bonvalet, 1983). We have recently shown that this receptor is also expressed in human epidermis, hair follicles and sweat glands (Kenouch et al., 1994). These results led us to investigate whether the subunits of the amiloride-sensitive sodium channel were expressed in rat skin, which contains both non-transporting epithelial cells (epidermal keratinocytes) and transporting epithelial cells (sweat glands).

The expression of the α, β and γ rENac subunits was demonstrated both by RNase protection assay and by in situ hybridization. They were expressed in sweat glands as well as in epidermal and follicular keratinocytes. Interestingly, the level of expression of each subunit mRNA varied in a cell-specific manner (e.g. the expression of the β subunit was less pronounced in the epidermis and in the hair follicle than in sweat glands). Furthermore the expression of the rENac mRNAs was less pronounced in small basal undifferentiated epidermal keratinocytes than in large differentiated suprabasal ones.

**MATERIALS AND METHODS**

**Animals**

Sprague-Dawley male rats (2-3 months old; Charles River, France) were anaesthetized with ether. For RNase protection assay, abdominal and sole skin samples were obtained, immediately frozen in liquid nitrogen and stored at -80°C until use. For in situ hybridization experiments animals were first perfused in the aorta with a solution of 4% paraformaldehyde in phosphate-buffered saline PBS (7 mM Na2HPO4·2H2O, 3 mM NaH2PO4·H2O, 150 mM NaCl, pH 7.4), and then full thickness abdominal and sole skin samples were obtained.

**RNase protection assay**

Total RNA was extracted from frozen rat skin or from kidney by a standard guanidium thiocyanate/phenol/chloroform extraction method (Chomczynski and Sacchi, 1987). The RNase-protection assay was performed as described (Sambrook et al., 1989). Antisense sodium channel cRNA probes were synthesized using a T3/T7 in vitro RNA synthesis kit (Promega) in the presence of [35S]UTP (Amersham, specific activity >15 TBq/mmol) from the 3¢ untranslated region of each rENac subunit cDNA. Three probes were obtained: α, 361 nt; β, 259 nt; γ, 511 nt and the expected protected fragments were 317, 206, and 443 nucleotides (nt), respectively. Samples of 100 µg of total RNA obtained from skin, 10 µg of total RNA from kidney, or 10 µg of yeast tRNA (Boehringer) were hybridized with 5×105 cpm radiolabelled probe in 80% formamide, 40 mM 1,4-piperazine-diethanesulphonic acid (Pipes, pH 6.4), 400 mM NaCl, 1 mM EDTA at 50°C overnight. RNase digestion (RNase A, 40 µg/ml and T1, 2 µg/ml from Boehringer) was then performed at 30°C for 60 minutes, followed by a proteinase K treatment (125 µg/ml, Boehringer) at 37°C for 30 minutes. Samples were phenol extracted, ethanol precipitated, and the protected fragments were separated by electrophoresis on a polyacrylamide gel. Gels were then fixed with 10% CH3COOH, vacuum dried, then exposed to Kodak X-OMAT AR 5 films.

**In situ hybridization**

The 3¢ untranslated region of the rat α, β and γ amiloride-sensitive sodium channel subunits cDNA (Canessa et al., 1993, 1994) were subcloned into Bluescript KS plasmids (corresponding to nt 2,185-2,775 for α, to nt 2,150-2,463 for β, and to nt 2,470-2,911 for γ subunit), and anti-sense and sense RNA probes were synthesized using a T3/T7 in vitro RNA synthesis kit (Promega) in the presence of [35S]UTP (Amersham, specific activity >37 TBq/mmol). The α, β and γ probes were 590, 313 and 441 nt long, respectively.

Skin samples were fixed in 4% paraformaldehyde for 6 hours, dehydrated in graded alcohol and embedded in paraffin. Sections of 5-7 µm were obtained and in situ hybridization was performed as previously described (Farman et al., 1991; Kenouch et al., 1994). Briefly, sections were rehydrated, treated with proteinase K, postfixed, acetylated, dehydrated and dried. Hybridization mix (50% formamide, 1 mM dithiothreitol (DTT)), 2× sodium saline citrate (SSC; 1× SSC is 0.15 M NaCl, 0.015 M trisodium citrate, pH 7.0), 10% dextran sulphate, 1 mg/ml salmon sperm DNA, and 35S-cRNA probe) was applied onto the sections which were then covered with parafilm, and exposed to a T3/T7 in vitro RNA synthesis kit (Promega) in the presence of [35S]UTP (Amersham, specific activity >15 TBq/mmol) from the 3¢ untranslated region of each rENac subunit cDNA. Three probes were obtained: α, 361 nt; β, 259 nt; γ, 511 nt and the expected protected fragments were 317, 206, and 443 nucleotides (nt), respectively. Samples of 100 µg of total RNA obtained from skin, 10 µg of total RNA from kidney, or 10 µg of yeast tRNA (Boehringer) were hybridized with 5×105 cpm radiolabelled probe in 80% formamide, 40 mM 1,4-piperazine-diethanesulphonic acid (Pipes, pH 6.4), 400 mM NaCl, 1 mM EDTA at 50°C overnight. RNase digestion (RNase A, 40 µg/ml and T1, 2 µg/ml from Boehringer) was then performed at 30°C for 60 minutes, followed by a proteinase K treatment (125 µg/ml, Boehringer) at 37°C for 30 minutes. Samples were phenol extracted, ethanol precipitated, and the protected fragments were separated by electrophoresis on a polyacrylamide gel. Gels were then fixed with 10% CH3COOH, vacuum dried, then exposed to Kodak X-OMAT AR 5 films.

**Fig. 1.** RNase protection assay of total RNA extracted from kidney, sole and abdominal skin. Samples were hybridized with α and β (left panel), or γ (right panel) 35S-cRNA probes, together with the GAPDH probe. The RNase-protected bands are indicated by the horizontal arrows. The GAPDH signal is also shown on the bottom of the figure after shorter exposure for the left gel. In each of these tissues, α, β and γ mRNAs were detected. However, each subunit was expressed at a lower level in skin than in kidney, and 100 µg total skin RNA was necessary compared with 10 µg total kidney RNA to obtain a clear signal. These probes did not interact with yeast tRNA, assessing the specificity of the signals observed in kidney and skin.
incubated overnight at 50°C. Post-hybridization washes consisted of a first wash (10 mM DTT, 5x SSC) (20 minutes at 50°C) followed by a high stringency wash (50% formamide, 2x SSC, 100 mM DTT) at 65°C for 20 minutes, and then by several washes in NaCl-Tris-EDTA (0.5 M NaCl, 10 mM Tris-HCl, 5 mM EDTA). An RNase A treatment (20 µg/ml) was then performed at 37°C for 20 minutes. Sections were washed several times in NaCl-Tris-EDTA then once in 0.1x SSC before they were dried. They were then covered with autoradiography emulsion (NTB 2 Kodak), and exposed at −20°C for 4-5 weeks before they were developed (Kodak D19). They were then stained with 0.05% toluidine blue and examined under bright-field and dark-field using a Zeiss Axiosplan microscope before they were photographed.

Quantification of the hybridization signal

Quantification of the hybridization signal was performed using an image analysing system (Optilab, Grafteck, France) on sections made from foot-pad epidermis obtained from 4 different animals. Sections were hybridized with α, β, γ antisense prob es and exposed for 13 days. For each section, three different fields were analyzed. For each field, a bright-field image was used to delineate an area of the basal layer (about 100 cells per delineated area), an area of the suprabasal layer (about 75 cells per area), and an equivalent area of the dermis. These delineated areas were superimposed on the dark-field image of the same zone and the optical density of the silver grains was measured to quantify the signal (as optical density per unit surface area).

A paired t-test was used to examine the difference in expression between the two skin areas.

RESULTS

Expression of the α, β and γ rENac subunits in full thickness rat skin

Total RNAs were extracted from full thickness skin obtained from the foot pad and the abdomen. Total RNAs were also extracted from kidney since the α, β and γ subunits of the amiloride-sensitive sodium channel are known to be expressed in the renal collecting duct (Canessa et al., 1993, 1994; Duc et al., 1994). The expression of the α, β and γ rENac subunit mRNAs was examined by RNase protection assay, and mRNAs specific for each subunit were detected in foot pad skin as well as in kidney (Fig. 1). In abdominal skin, only α and γ signals were visible. Although these experiments were not designed to examine quantitatively the relative expression of rENac subunit mRNAs in the kidney and skin, it should be noted that it was necessary to load 100 µg of RNA from skin to visualize the signals, while 10 µg of RNA from kidney was sufficient. Because of the cellular heterogeneity of these tissues, it is difficult to compare directly the organ-specific levels of expression of these mRNAs.

To examine further the expression of the α, β and γ subunits of the amiloride-sensitive sodium channel in glabrous and hairy skin, histological sections were obtained from foot pad (sole) and from abdominal skin. Sense and antisense RNA probes specific for each rENac subunit were synthesized, and in situ hybridization was then performed as described.

Expression in epidermis

The α, β and γ subunits were expressed in the epidermis and in the eccrine sweat glands of the foot pad, compared with dermal expression which was at background level (Fig. 2). The three rENac subunits were expressed in all epidermal layers, but α and γ expression was prevalent over that of the β subunit (Figs 2 and 3). However, it should be noted that some unspecific hybridization signal was obtained when control sections were hybridized with the α sense probe (Fig. 3D). Nevertheless, the signal obtained with this probe was always lower than that obtained with the α antisense probe. The reason for the existence of a higher background level with the α sense probe over the β and γ sense probes is unknown.

Microscopic examination of the sections suggested that there might exist variations in the expression of the α, β and γ subunits depending upon the state of differentiation of the epidermal cells. To examine this hypothesis further, we compared the expression of the different rENac subunits in proliferative basal cells to that of differentiated suprabasal cells. For this purpose, sections from foot pads were hybridized with antisense α, β and γ subunit probes and were exposed for a shorter time (13 days) to allow for a gross quantification of hybridization signals using an image analysing system. Fig. 3 illustrates the hybridization signals observed in this condition (13 days exposure) in the epidermis, with α, β and γ antisense and sense probes. Control sense probes gave a low and uniform signal, while antisense probes showed a concentration of silver grains predominating in the suprabasal cells, as compared with the proliferative basal cells. Quantification of this differential expression (Fig. 4) shows that the estimated signals that were obtained for each rENac subunit were weaker in small basal cells than in large differentiated suprabasal cells. For each subunit, this difference was statistically significant (P<0.02 for α and γ, and P<0.05 for β subunit, paired t-test; n=4 rats). This result suggests that the amiloride-sensitive sodium channel could be differentially expressed depending upon the state of differentiation of the keratinocyte.

Expression in sweat glands

α, β and γ rENac subunit mRNAs were also expressed in sweat glands located in the dermal part of the foot pads (Figs 2 and 5). However, the expression of β and γ subunits was higher than that of α subunit.

Expression in hair follicles

The α, β and γ subunits were clearly expressed in the epithelial cells of the hair follicle (Fig. 6). However, it was difficult to determine precisely if they were expressed either in the cells constituting the outer epithelial sheath or the inner sheath, or in both. Interestingly, the level of expression of each subunit appeared again to be different, α and γ subunits showing the strongest expression (Fig. 6A and C), and β subunit the lowest (Fig. 6B). No significant expression of the different subunits was detected in the dermis. The expression in the follicular papilla was not determined since hair follicles were sectioned transversely above the papillae.

DISCUSSION

We report that mRNAs encoding the α, β and γ subunits of the amiloride-sensitive sodium channel (rENac) are expressed in rat skin, namely in epidermis, hair follicles and in sweat glands. These subunits of the rENac are usually expressed in tight epithelia which can reabsorb and transport sodium as the
Fig. 2. In situ hybridization of rat sole skin with $\alpha$ (A,D), $\beta$ (B,E), and $\gamma$ (C,F) sodium channel cRNA antisense probes. Each field has been photographed as bright field (A,B,C) and dark field (D,E,F) of the same zone. The epidermal layers exhibited a strong signal with $\alpha$ probe; much less labelling was apparent with $\beta$ and $\gamma$ probes. Conversely, sweat glands expressed a much higher signal with $\beta$ and $\gamma$ than with the $\alpha$ probe. Dermis was negative as well as blood vessels. Bar, 100 $\mu$m.

Fig. 3. In situ hybridization of rat sole skin epidermis with $\alpha$ (A), $\beta$ (B) and $\gamma$ (C) antisense or $\alpha$ (D), $\beta$ (E) and $\gamma$ (F) sense probes. Exposure time was reduced to 13 days to evidence the different expression in the basal palissadic cells of the epidermis ($\Delta$) and in the mid-layer cells ($\ast$) of much larger size. For $\alpha$ and $\gamma$ subunits, the signal observed with the control sense probe was much lower than that observed with the antisense probe. The signal obtained with $\beta$ antisense probe was low as compared with the $\alpha$ and $\gamma$ antisense signal. Bar, 10 $\mu$m.
Sodium channel subunit mRNAs in skin

Cortical collecting ducts of kidney, the distal colon, and the ducts of salivary glands (Duc et al., 1994). RNase protection assay demonstrated the presence of those subunit mRNAs in both abdominal and sole skin samples. This is in agreement with a previous report which reported a faint expression of α subunit mRNA in skin (Li et al., 1994).

**Fig. 4.** Quantification of the hybridization signals observed within the epidermis of the sole skin. Sole skin sections were hybridized with α, β and γ antisense probes, as in Fig. 3. For each probe, quantification (i.e. optical density per surface area, in arbitrary units) was performed over the basal layer of the epidermis (A) indicated by △ on Fig. 3, and over the large cells (B) of the same section, indicated by * on Fig. 3. Each measurement was done on three different fields for each rat; four rats were used in each study. Symbols refer to mean values (± s.e.m.) of each rat. Lines join paired values in A (basal cells) and B (differentiated cells) determined on the same field. Differences (paired t-test) in hybridization signal between these two cell types were significant for each subunit (P<0.02 for α and γ, and P<0.05 for β subunit).

**Fig. 5.** In situ hybridization of rat sole skin sweat glands with α (A,D), β (B,E) and γ (C,F) sodium channel cRNA antisense (A,B,C) and sense (D,E,F) probes. The specific hybridization signal observed with the antisense probes (A for α, B for β, and C for γ probe) was much higher with β and γ than with α probe. A low signal was found using the sense probes (D for α, E for β, and F for γ). Bar, 10 μm.

**Fig. 6.** In situ hybridization of rat abdominal skin with α, β, and γ sodium channel cRNA antisense (A,B,C) and sense (D,E,F) probes. The antisense probe showed essentially expression of α and γ subunit mRNAs in the hair follicles, while dermis was negative; a very low signal was observed with β probe. Sense probe hybridization showed a low, non specific signal over the same structures. Bar, 25 μm.
The expression of the rENaC subunits in epidermal keratinocytes as well as in hair follicles is quite unexpected. However, it is not surprising that the rENaC subunits are expressed in sweat glands, primarily in the duct and, to a lesser extent, in the glandular coil since reabsorption of sodium is known to occur. This reabsorption of sodium is amiloride-sensitive, and sodium conductance has been demonstrated at the apical pole of the luminal ductal cells (Quinton, 1981; Sato et al., 1988; Joris et al., 1989). This process contributes to the control of extracellular sodium and fluid levels, particularly in situations in which adjustment to heat is required (Conn, 1963). This sodium reabsorption is sensitive to the mineralocorticoid hormone, aldosterone (Conn, 1949, 1963; Sato, 1977), and the presence of mineralocorticoid receptors has been demonstrated recently in these cells (Kenouch et al., 1994). The enzyme 11β hydroxysteroid dehydrogenase which ensures selective mineralocorticoid binding and action, is also present in these cells (Kenouch et al., 1994). Therefore, our finding that α, β and γ subunit mRNAs of the amiloride-sensitive sodium channel (rENaC) are present in sweat gland ducts is in agreement with these previous data. That these subunits, mostly β and γ, are also present in the sweat gland coils, is more surprising since sweat formation results primarily from the secretion of an isotonic fluid, presumably through a Na+·K+·2Cl− transport system (Sato et al., 1989). However, only a limited inhibitory effect of amiloride on basolateral membrane potential of sweat secreting cells, which could be attributed to an interaction with a sodium channel or a sodium-proton exchanger, has been reported (Sato and Sato, 1987). On the other hand, amiloride did not affect the rate of sweat secretion (Quinton, 1981), and no specific staining was found when primary cultures of coil epithelial cells were stained with a sodium channel antibody (Tousson et al., 1989). Also amiloride did not inhibit cation conductance in cultivated human secretory sweat gland cells (Krouse et al., 1989). Nevertheless, mineralocorticoid receptors were demonstrated in either the coil of human sweat glands or in initial parts of their ducts. It is also possible that the β and γ subunits may be part of another channel, not amiloride-sensitive, requiring the presence of another α subunit isoform. Altogether, these observations indicate that more studies are required to demonstrate that there exists a functional amiloride-sensitive sodium channel in coil epithelial cells of sweat glands and that it is regulated by aldosterone.

The epidermis is a pluristratified squamous epithelium, mostly composed of keratinocytes which maintain its structure and cohesion. Keratinocytes located in the epidermal basal layer can multiply and are responsible for the renewal of the epithelium. However, they undergo programmed terminal differentiation after leaving the basal layer and they become postmitotic. As a result of this program, anucleated and cornified cells are formed, and these squames will be later eliminated (for review see Fuchs, 1990).

To our knowledge, epidermal and hair follicle keratinocytes are not known to transport sodium, even if mineralocorticoid receptors were recently demonstrated in these cells (Kenouch et al., 1994). Whether aldosterone could modulate a sodium channel activity in keratinocytes is still unknown. However, our finding that α, β and γ subunit mRNAs of the amiloride-sensitive sodium channel were expressed in rat epidermal and follicular keratinocytes strengthens this hypothesis, but its role needs to be established. It is quite possible that it is involved in the control of cell volume (Häussinger and Lang, 1992) which is thought to play an important role during epidermal proliferation and differentiation (Bergstresser et al., 1978; Watt and Green, 1982; Barrandon and Green, 1985). Interestingly, α, β and γ subunit mRNAs are less abundant in the small proliferative basal keratinocytes than in that of the large differentiated suprabasal keratinocytes. Whether sodium channel expression is related to the larger cell volume of suprabasal keratinocytes as compared with that of basal keratinocytes remains to be explored, particularly in relation to cell differentiation. The use of cell cultures should help to gain some insight into the role of the Na channel in keratinocyte differentiation.

Finally α, β and γ subunit mRNAs of the amiloride-sensitive sodium channel were expressed at different levels depending on the location of the epithelial cells in the skin, e.g. α and γ subunit were prevalent in epidermis and hair follicle as β and γ were prevalent in sweat glands (Table 1). This is quite unexpected since these subunits are normally expressed at a similar level in sodium transporting epithelia such as the renal collecting duct (Duc et al., 1994). It is possible that particular associations e.g. βγ in sweat glands and αγ in keratinocytes result in different functional properties, or that they associate with other subunits which are still uncharacterized.

In conclusion, we have shown that α, β and γ subunit mRNAs of the epithelial sodium channel rENaC are expressed in rat skin. Two unexpected observations were made: (1) their expression is dissociated unlike that of tight epithelia (kidney collecting duct, distal colon...). this suggests a different stoichiometry of the channel protein possibly related to its function. (2) Their expression is more pronounced in the suprabasal layers of the epidermis than in the indifferntiated basal cell layer.

We thank B. C. Rossier and C. Canessa for the rENaC probes and for helpful discussions; M. Paulson for critical reading of the manuscript; and V. Lévéque for expert typing.

REFERENCES


Table 1. Relative expression of rENaC subunit mRNAs in skin structures and kidney

<table>
<thead>
<tr>
<th>Subunit</th>
<th>Hair follicle</th>
<th>Epidermis</th>
<th>Sweat glands</th>
<th>Kidney collecting tubule</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>β</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>γ</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

In situ hybridization was performed on sections of abdominal and sole skin. Since these two tissues were placed on the same slide, comparisons were made on the relative level of expression of each subunit mRNA over skin structures. These data are compared to those obtained in similar experimental conditions in the kidney collecting tubule (from Duc et al., 1994).
Sodium channel subunit mRNAs in skin


(Received 22 March 1995 - Accepted 12 November 1995)