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Occurrence of NaK-ATPase isoforms during rat inner ear development and functional implications

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Abstract This study examined the presence of NaK-ATPase isoforms in the developing inner ear of the rat and studied the importance of functional subunit combinations in endolymph homeostasis. The findings were: (a) the combination $\alpha_1\beta_1$ is found in epithelial, mesenchymal, and neural inner ear cells with an early starting expression 14 days postconception (dpc) in some endolymphatic sac cells; (b) from 1 day after birth (dab) expression of $\alpha_1\beta_2$ is observed in marginal cells, vestibular dark cells, and certain vestibular nonsensory cells; (c) a transient expression of $\alpha_2\beta_1$ is found in suprastrial fibrocytes and spiral ligament fibrocytes type II between 10 and 15 dab; (d) starting at 16 dpc the combination $\alpha_3\beta_1$ is uniquely expressed in inner ear neural cells (as in other neural tissues). In conclusion, during development a switch from $\alpha_2\beta_1$ towards $\alpha_1\beta_1$ is observed in suprastrial fibrocytes and in spiral ligament fibrocytes type II. Thus, according to the biochemical characteristics of these combinations, a switch towards a NaK-ATPase with higher capacity takes place. In addition, prominent expression of the $\alpha_1\beta_2$ combination in predominantly K^+ ion transporting marginal and dark cells is in accordance with the characteristic of this combination and thus with the presumed function of these cells as important K^+ suppliers for the endolymph. We believe this combination in certain vestibular nonsensory cells to be involved in K^+ sensing. Early expression of the $\alpha_1\beta_1$ combination in the endolymphatic sac, prior to that in the other parts of the inner ear, suggests that this structure may be involved to some extent in the development of the vestibulum and cochlea.

Keywords Endolymph · NaK-ATPase · Inner ear · Development · Rat

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Introduction

Endolymph, characterized by an unique intracellularlike ionic composition, plays a crucial role in the normal functioning of the inner ear. Changes in endolymph composition eventually lead to impaired hearing. NaK-ATPases that normally couple the hydrolysis of one molecule ATP to the transport of three Na^+ ions out and two K^+ ions into the cell are involved in endolymph ion-exchange processes [13]. NaK-ATPases are composed of a transporting α and stabilizing β subunit. For their biological activity both subunits are needed [11]. The β subunit affects the affinity of the α subunit for both Na^+ and K^+ ions [1, 2]. In mammals three well characterized isoforms of the α subunit (α_1, α_2, and α_3) and at least two of the β subunit (β_1 and β_2) have been described [24]. Schmalzing et al. [22] convincingly demonstrated differential interaction between different α and β isoforms. In different tissues specific combinations are found. Thus, such tissue-specific combinations may determine a specific function. Both α_1 and β_1 isoforms are ubiquitous in most tissues and are at present the only isoforms detected in the kidney [9]. The α_1 and β_2 isoforms are present in ion transporting tissues such as the choroid plexus [30] and pigmented retinal epithelium [20]. In excitable tissues such as muscles and nerves the isoforms α_2, α_3, β_1, and β_2 are predominantly expressed [12, 16].

In the inner ear the following distribution of individual NaK-ATPase isoforms has been found. ten Cate et al. [4] and McGuirt and Schulte [18] described the unique expression of α_1 and β_2 in dark and marginal cells and of α_3 and β_1 in neural elements in adult rat and gerbil, respectively. Both groups reached the overall conclusion that differential expression of NaK-ATPase probably reflects functional diversity. The expression of α_1 and β_2 in the adult guinea pig endolymphatic sac led ten Cate et al. [5] to the suggestion that this combination reflects a distinct inner ear NaK-ATPase.

Studies on NaK-ATPase expression during inner ear development have been performed only in the cochlea of

the gerbil [19], rat [31], and mouse [8]. No attention has been paid to the development of the vestibular portion. Despite the important function ascribed to the endolymphatic sac and duct in endolymph homeostasis no data are available on expression of the various NaK-ATPase isoforms during the development of these structures.

In this developmental study we compared the expression of NaK-ATPase subunits in endolympatic sac and duct with those in cochlea and vestibulum. In addition, recent biochemical studies [2, 7] on characteristics of NaK-ATPase isoform combinations enabled us to hypothesize on the involvement of distinct isoform-combinations in endolymph homeostasis.

Materials and methods

Animals

Wistar rats (own breeding facility) were housed in standard cages and received water and food ad libitum. Animal experiments were conducted in accordance with international guidelines.

Immunohistochemistry

The expression of NaK-ATPase isoforms was investigated during the development of the rat inner ear from 14 days postconception (dpc) to 30 days after birth (dab). Investigated time points were 14, 16, and 18 dpc and 1, 4, 10, 15, 20, and 30 dab. Anti-NaK-ATPase antibodies used in this study: mouse anti-rat monoclonal antibodies against α_1 and α_2 chain (McK1 and McB2; Prof. Dr. K. J. Sweadner, Boston, Mass., USA) and rabbit anti-rat polyclonal antibodies against α_3, β_1, and β_2 chain (UBI, New York, N.Y., USA).

Table 1 Expression of functional NaK-ATPases in different cells during inner ear development

	14	16	18	Birth	1	4	10	15
$\alpha_1\beta_1$								
Cochlea								
External sulcus cells					■	■	■	■
Marginal cells					■	■	■	■
GER cells					■			
Root cells						■	■	■
Interdental cells							■	■
Claudius cells								■
Reissner's membrane cells								■
Suprastrial fibrocytes								■
Spiral ligament fibrocytes type II								■
Spiral ligament fibrocytes type IV								■
Coclear neurons								■
Vestibulum								
Nonsensory cells			■	■	■	■	■	■
Dark cells			■	■	■	■	■	■
Supporting cells			■	■	■	■	■	■
Transitional cells						■	■	■
Vestibular neurons								■
Endolymphatic sac/duct								
Endolymphatic sac cells	■	■	■	■	■	■	■	■
Endolymphatic duct cells						■	■	■
$\alpha_1\beta_2$								
Cochlea								
Marginal cells[a]					■	■	■	■
Vestibulum								
Nonsensory cells[b]					■	■	■	■
Dark cells					■	■	■	■
$\alpha_2\beta_1$								
Cochlea								
Supralimbal fibrocytes							■	■
Suprastrial fibrocytes							■	■
Spiral ligament fibrocytes type II							■	■
$\alpha_3\beta_1$								
Cochlea								
Cochlear neurons		■	■	■	■	■	■	■
Vestibulum								
Vestibular neurons		■	■	■	■	■	■	■

[a] Intensification of staining α_1 and β_2 between 4 and 15 dab
[b] No staining of β_2 in some ampullar nonsensory cells

Cryostat sections were used for monoclonal antibodies, and Bouin-fixed paraffin sections were used for polyclonal antibodies. At least four animals of every age category were examined. Decalcification procedure was performed as described before [15].

Immunostaining with monoclonal antibodies was performed according to the previously described immunoperoxidase method [28]. Briefly, cryostat sections were fixed in cold (4 °C) acetone for 10 min, rinsed in phosphate-buffered solution (PBS), and subsequently incubated at room temperature for 60 min with either anti-α_1 (1 : 10) or anti-α_2 (1 : 10) antibody. After rinsing in PBS the sections were incubated for 30 min with peroxidase-conjugated rabbit anti-mouse IgG (Dakopatts, Glostrup, Denmark), and peroxidase activity was detected after a 10 min incubation with 3′-amino-9-ethylcarbozole as substrate.

Immunostaining with polyclonal antibodies was performed by use of the avidin/biotin complex technique. Paraffin sections were deparaffinized in toluol and rehydrated. For α_3 detection sections were pretreated with 0.1% trypsin/0.2% $CaCl_2$ in PBS for 10 min at 37 °C. All sections were placed in 0.5 M NH_4Cl in PBS containing 0.4% Triton X-100 (TPBS) for 40 min. After rinsing in TPBS they were preincubated for 40 min with 1.5% normal goat serum/1% bovine serum albumin (BSA) in TPBS. Incubation of the sections with anti-α_3, anti-β_1 or anti-β_2 (diluted 1 : 100, 1 : 200, and 1 : 500 in TPBS/1% BSA respectively) was performed overnight at 4 °C. After rinsing in TPBS sections were incubated for 60 min with biotin-labeled goat anti-rabbit IgG (Dakopatts) diluted 1 : 400 in TPBS/1% BSA. The next incubation, after rinsing with TPBS, was performed with peroxidase-labeled streptavidin (50 ng/µl in TPBS; Dakopatts) for 90 min. After rinsing in PBS, followed by rinsing in sodium-acetate buffer (0.05 M, pH 4.9), peroxidase activity was determined by a 10 min incubation with 3′-amino-9-ethylcarbozole.

All cryostat and paraffin sections were counterstained with Mayer's hemalum and mounted in glycerin jelly. Control sections were incubated with nonimmune mouse serum.

Results

The expression of NaK-ATPase α and β isoforms as determined by immunohistochemistry is reported separately for the developing cochlea, vestibulum, and endolymphatic sac and duct. Table 1 lists the expression of functional NaK-ATPases in different cells during inner ear development. In addition, Figs. 1 and 2 schematically depicts the expression of NaK-ATPase α and β isoforms in adult cochlear (Fig. 1) and vestibular (Fig. 2) structures.

Cochlea

Maturation of the cochlea occurs via a base to apex gradient. All results described apply to the basal turn.

α_1

At 1 dab expression can be distinguished in external sulcus cells, basal part of marginal cells, and in some GER cells. Between 4 (Fig. 3 A) and 15 dab (Fig. 3 D) intensification of staining is observed in marginal cells; root cells (Fig. 3 A) begin to stain at 4 dab. Starting at 10 dab α_1 expression is seen in interdental cells and at 15 dab in Claudius cells, Reissner's membrane, spiral limbus fibrocytes, suprastrial fibrocytes (Fig. 3 D; Fig. 4 A, D), and spiral ligament fibrocytes type II (Fig. 3 D; Fig. 4 F) and

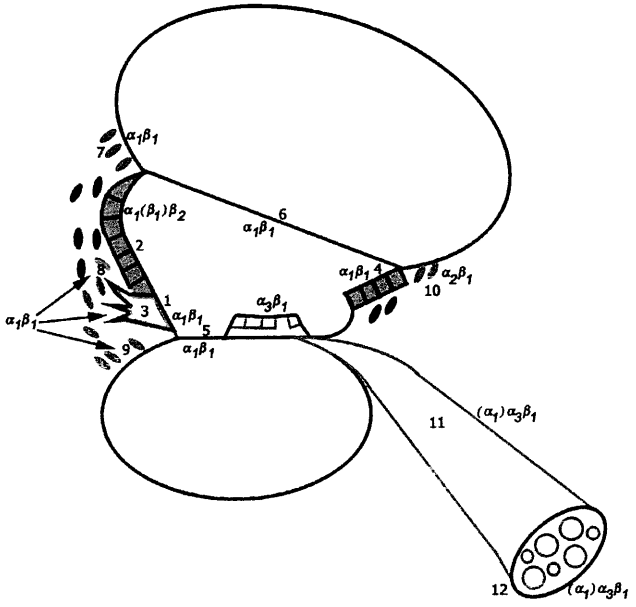

Fig. 1 Schematical overview of the presence of the different NaK-ATPase α and β isoforms expressed in the adult cochlear duct. *1* External sulcus; *2* marginal cells; *3* root cells; *4* interdental cells; *5* Claudius cells; *6* Reissner's membrane; *7* suprastrial fibrocytes; *8* spiral ligament fibrocytes type II; *9* spiral ligament fibrocytes type IV; *10* supralimbal fibrocytes; *11* cochlear nerve; *12* spiral ganglion; *in parenthesis* no prominent expression

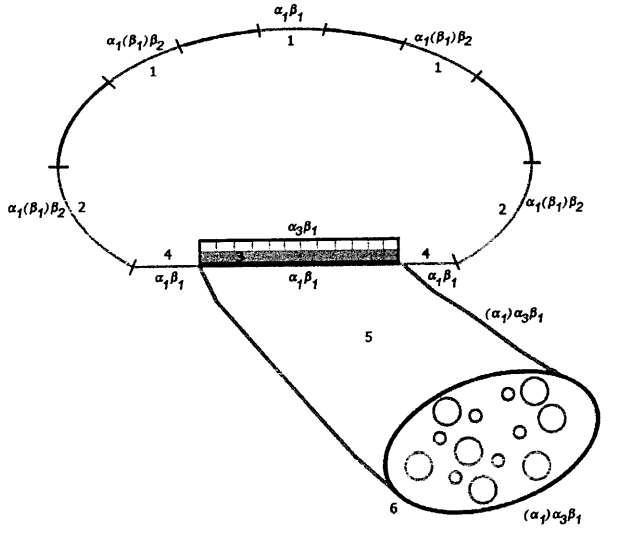

Fig. 2 Schematical overview of the presence of the different NaK-ATPase α and β isoforms expressed in the adult vestibular portion. *1* Nonsensory cells; *2* dark cells; *3* supporting cells; *4* transitional cells; *5* vestibular nerve; *6* vestibular ganglion; *in parenthesis* no prominent expression

IV (Fig. 3 D). Cochlear neurons are also positive from 15 dab on.

α_2

From 4 dab on expression is found in spiral limbus fibrocytes, supralimbal fibrocytes and perineural cells. Expres-

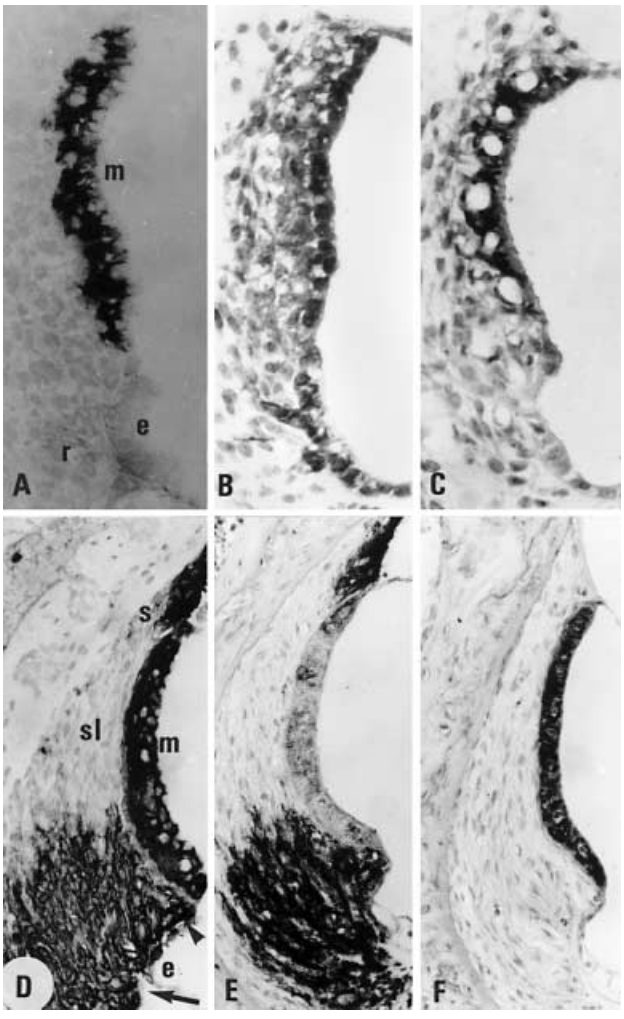

Fig. 3 Immunohistochemical staining of different NaK-ATPase isoforms in marginal cells *(m)* at 4 dab for α_1 (**A**), β_1 (**B**), and β_2 (**C**); at 15 dab for α_1 (**D**), β_1 (**E**), and β_2 (**F**). Note in the marginal cells intensification of staining for α_1 and β_2 between 4 and 15 dab and the change of a weak general to a more scattered staining of β_1. *e* External sulcus; *r* root cells; *s* suprastrial fibrocytes; *sl* spiral ligament; *arrowhead* spiral ligament fibrocytes type II; *arrow* spiral ligament fibrocytes type IV. **A**–**F** × 200

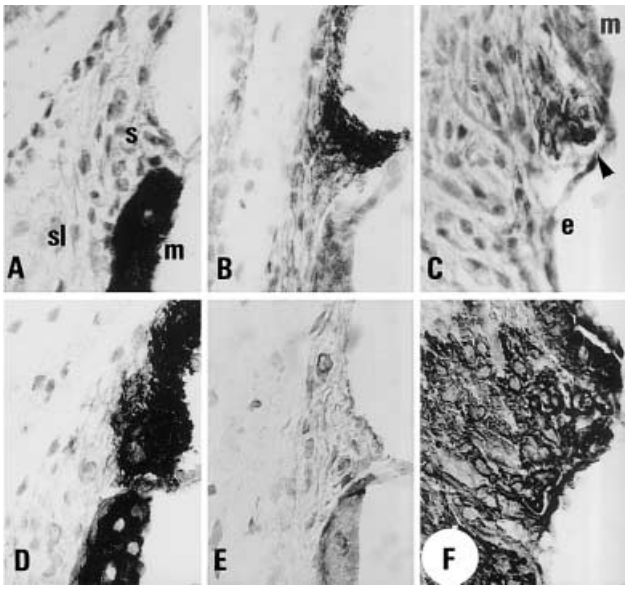

Fig. 4 Immunohistochemical staining of different NaK-ATPase isoforms in suprastrial fibrocytes(s) (**A**,**B**,**D**,**E**) and spiral ligament fibrocytes type II (*arrowhead*; **C**,**F**) at 10 (**A**–**C**) and 15 dab (**D**–**F**) for α_1 (**A**,**D**,**F**) and α_2 (**B**,**C**,**E**). Note that from 10 to 15 dab staining of α_2 disappears and prominent staining of α_1 occurs. *e* External sulcus; *m* marginal cells; *sl* spiral ligament. **A**–**F** × 400

sion of α_2 in suprastrial fibrocytes (Fig. 4B) and spiral ligament fibrocytes type II (Fig. 4C) appears at 10 dab and begins to disappear from 15 dab (Fig. 4E). From 15 dab expression is observed in strial basal cells.

α_3

From 16 dpc on staining is visible in the cochlear neurons.

β_1

From 16 dpc expression is found in the cochlear neurons. Positivity of β_1 is seen from 18 dpc in the external sulcus cells, marginal cells, GER, LER, and Reissner's membrane. Staining in marginal cells develops from a weak general signal at 4 dab (Fig. 3B) to a more scattered one at 15 dab (Fig. 3E). At 4 dab staining of the root cells starts (Fig. 3B) and from 10 dab on β_1 expression is present in the interdental cells, Claudius cells, supralimbal fibrocytes, suprastrial fibrocytes (Fig. 3E) and spiral ligament fibrocytes types II and IV (Fig. 3E).

β_2

Only marginal cells show expression from 1 dab on (Fig. 3C) with an intensification until 15 dab (Fig. 3F).

Vestibulum

α_1

From 18 dpc the nonsensory cells show heterogeneous staining. An intense reaction is found in the ampullar region. Dark cells of cristae and macula stain faintly at this age. A positive α_1 reaction is found in the supporting cells of both maculae whereas a less prominent reaction is found in the cristae. From 1 dab on the dark cells (Fig. 5A) of cristae and macula stain intensely, and from 4 dab on the transitional cells (Fig. 5B) are positive. Vestibular neurons are labeled from 15 dab on.

α_2

From 4 dab staining of α_2 is observed in the fibrocytes below the vestibular epithelial cells.

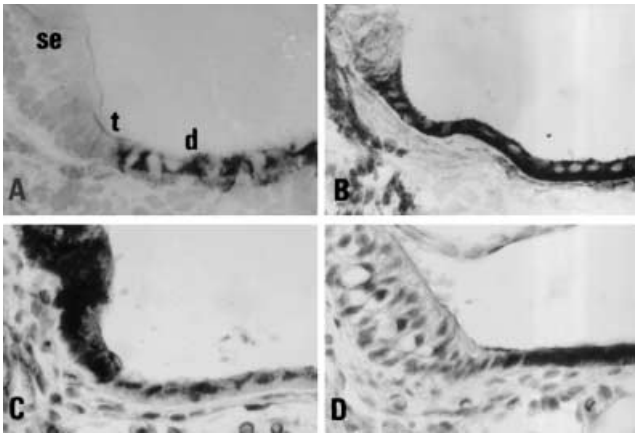

Fig. 5 Immunohistochemical staining of different NaK-ATPase isoforms at 1 (**A**) and 15 dab (**B**–**D**) in dark cells *(d)* for α_1 (**A**,**B**), β_1 (**C**) and β_2 (**D**) and in transitional cells *(t)* that only stained for α_1 (**B**) and β_1 (**C**). *se* Sensory cells. **A** × 200; **B**–**D** × 400

α_3

From 16 dpc expression occurs in the vestibular neurons.

β_1

From 16 dpc a positive β_1 reaction is observed in the vestibular neurons and sensory epithelial cell areas. At 18 dpc heterogeneous staining becomes apparent in the nonsensory cells and dark cells. In addition transitional cells (Fig. 5 C) show intense staining. The supporting cells are also positive from this stage.

β_2

Some nonsensory cells and dark cells (Fig. 5 D) stain intensely from 1 dab on.

Endolymphatic sac and duct

In these structures only α_1 and β_1 are found. They are expressed very early in comparison with the cochlear and vestibular structures. Staining in saccus epithelium precedes staining in ductus epithelial cells.

α_1

The epithelial lining of the saccus shows heterogeneous expression from 14 dpc (Fig. 6 A, D). This pattern does not change throughout the observation period. After birth expression of α_1 intensifies (Fig. 6 E). Ductus cells start staining at 4 dab.

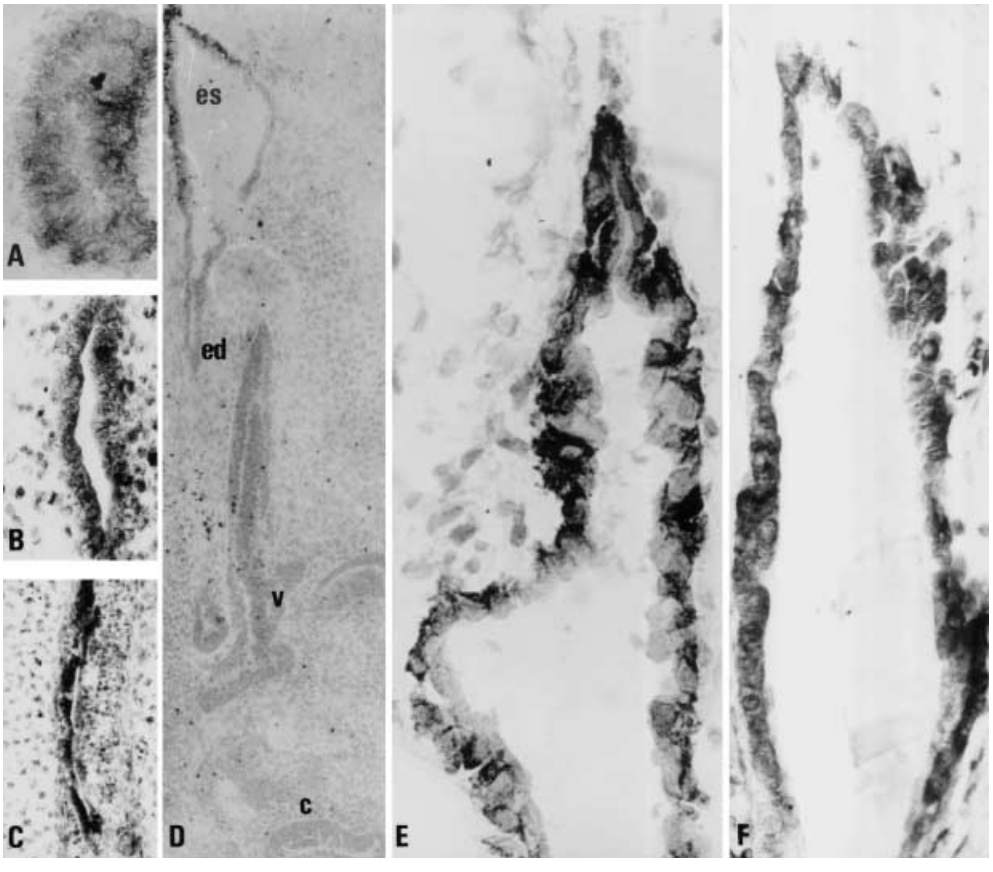

Fig. 6 Immunohistochemical staining of the NaK-ATPase isoforms α_1 (**A**,**D**,**E**) and β_1 (**B**,**C**,**F**) in the endolymphatic sac (**A**, **B**,**D**–**F**) and duct (**C**) at 14 dpc (**A**,**B**), 16 dpc (**D**), 18 dpc (**C**), and 15 dab (**E**,**F**). Note in **D** early heterogeneous staining in the endolymphatic sac *(es)* in contrast to the endolymphatic duct *(ed)*, vestibular portion *(v)* and cochlear duct *(c)*. **A**–**C**,**E**,**F**: × 200; **D** × 50

β_1

From 14 dpc the saccus epithelium shows heterogeneous expression of β_1 (Fig. 6 B, F). Expression in ductus cells starts at 18 dpc (Fig. 6 C).

Discussion

Since functional NaK-ATPases are always composed of an α and β subunit [7], we consider that cells only expressing one subunit are void of functional NaK-ATPases. However, we cannot exclude the involvement of noninvestigated isoforms.

The present observations demonstrate that the $\alpha_1\beta_1$ combination is found in epithelial, mesenchymal, and neural cells. In addition, $\alpha_1\beta_2$ is found in epithelial cells, $\alpha_2\beta_1$ in mesenchymal cells, and $\alpha_3\beta_1$ in neural cells. Several studies have confirmed that distinct NaK-ATPase isoform combinations determine their affinities for Na^+ and K^+ ions [1, 24]. Recently Crambert et al. [7] described the characteristics of human NaK-ATPase combinations whereas Blanco and Mercer [2] described those of the rat. The fact that a given combination may have different properties depending on the cellular environment must be taken into consideration as well [27]. Nevertheless, the combination $\alpha_1\beta_1$ displays a high K^+ and Na^+ affinity as well as a high turn-over rate; $\alpha_1\beta_2$ is characterized by a high K^+ affinity and a very high turnover rate, but its affinity for Na^+ has not been determined; $\alpha_2\beta_1$ has a moderate K^+ and Na^+ affinity and a moderate turnover rate. Finally, $\alpha_3\beta_1$ has a high K^+ and a low Na^+ affinity and a low turnover rate. This last combination differs in K^+ affinity between rat and human, i.e., K^+ affinity is lower in the rat. However, Therien and Blostein [26] also found a high K^+ affinity of $\alpha_3\beta_1$ in the rat with K^+ acting as a competitive reversible inhibitor of Na^+ affinity. Crambert et al. [7] concluded that combinations with α_1 isoforms perform housekeeping roles whereas combinations with α_2 or α_3 play a role in restoring resting conditions. Thus different isoform combinations exhibit major differences in transport capacities and thus may have important physiological consequences. Based on these characteristics we evaluate the role of functional NaK-ATPases in endolymph homeostasis.

During ontogenesis of rat inner ear epithelial cells the first NaK-ATPase isoforms that can form a functional combination are found in some endolymphatic sac cells, followed by distinct populations of vestibular and cochlear cells. This tendency in differential functional maturation is in accordance with the morphological development of these structures [15].

In inner ear neural tissue the unique expression of $\alpha_3\beta_1$ NaK-ATPase (high K^+ and low Na^+ affinity) is in line with other neural tissues as well as with its presumed function of restoring K^+ concentrations after depolarization. $\alpha_1\beta_1$ NaK-ATPase (high K^+ and Na^+ affinity) is also present in those neural cells and is likely to be involved in normal cell physiology.

A remarkable observation during the inner ear development is the expression switch from functional $\alpha_2\beta_1$ NaK-ATPases towards $\alpha_1\beta_1$ in the cochlear fibrocytes between 10 and 15 dab. Previous studies [19, 23] have suggested that these fibrocytes are involved in the transport of K^+ ions into the intrastrial region. Additionally, it has been proposed that those fibrocytes play an important role in the generation of the endocochlear potential which reaches adult values in the rat between 11 and 16 dab [3, 21]. This coincides with the rapid increase in NaK-ATPase activity in the stria vascularis to mature values [14]. The switch from $\alpha_2\beta_1$ to $\alpha_1\beta_1$ in suprastrial fibrocytes and in spiral ligament type II fibrocytes also occurs in this period. Because $\alpha_2\beta_1$ has a moderate K^+ and Na^+ affinity and moderate turnover rate whereas $\alpha_1\beta_1$ has a high K^+ and Na^+ affinity and high turnover rate, the switch towards the high-capacity NaK-ATPase during the final stages of inner ear maturation suggests a crucial role of $\alpha_1\beta_1$ in the maintenance of the adequate endolymph composition/endocochlear potential.

There is convincing evidence that vestibular dark cells and cochlear marginal cells transport K^+ from the basolateral to the apical side of the cell. K^+ secretion via an apical K^+ channel entails uptake of K^+ across the basolateral membrane via NaK-ATPase as well as a Na-K-Cl cotransporter. The Na^+ taken up via the basolateral Na-K-Cl cotransporter also increases the activity of the basolateral membrane NaK-ATPase, resulting in an additional flux of K^+ into the cell [29]. As in former cochlear and vestibular immunohistochemical studies [4, 19, 31] we detected the isoform combinations of $\alpha_1\beta_1$ and predominantly $\alpha_1\beta_2$ in marginal and dark cells. We hypothesize that the prominent presence of $\alpha_1\beta_2$ (high K^+ affinity and high turnover rate) in those specific K^+ ion transporting epithelia is required for maintaining the unique high K^+ and low Na^+ concentrations in the endolymph. This is supported by the observation that, in addition to $\alpha_1\beta_1$ NaK-ATPase, expression of $\alpha_1\beta_2$ is further enhanced between 4 and 15 dab (especially in marginal cells), the same period in which the endolymph K^+ concentration reaches mature values [3]. Additional support for this hypothesis may be derived from studies on β_2-deficient mice. These mutants display a disturbed cerebral ionic homeostasis [17].

In the vestibular part $\alpha_1\beta_2$ is found together with $\alpha_1\beta_1$ in distinct nonsensory cells, which are thought to prevent the local passive leakage of Na^+ ions into and K^+ ions out of the endolymphatic space [10]. We hypothesize that $\alpha_1\beta_2$ in these vestibular cells is involved in K^+ sensing and therefore reacts adequately on leakage-induced changes in endolymph composition.

The exact mechanisms involved in endolymph homeostasis in the endolymphatic sac are still obscure. The relative high Na^+ and low K^+ concentrations in the endolymphatic sac endolymph [6] suggests that the epithelium is involved in absorption of K^+ and secretion of Na^+. Absorption of K^+ may be explained by the presence of apical Na-K-Cl cotransporters [25], but this does not explain the high Na^+ concentration. It is tempting to assume that in the adult endolymphatic sac $\alpha_1\beta_1$ NaK-ATPase generates

a local electrochemical gradient that is an important factor in the process of absorption and secretion of solutes and electrolytes. In the light of the current opinion on the role of the endolymphatic sac in endolymph homeostasis the expression of $\alpha_1\beta_1$ NaK-ATPase in the sac prior to that in the duct and vestibular and cochlear parts is quite remarkable. It may suggest that this structure is involved in the development of the remaining part of the inner ear.

In conclusion, the present data together with reevaluation of the current biochemical data lead to a better understanding of the specific involvement of different inner ear cell types in endolymph homeostasis.

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References

1. Béguin P, Hasler U, Beggah A, Geering K (1998) Regulation of expression and function by subunits of oligomeric P-type ATPases. Acta Physiol Scand 163:283–287
2. Blanco G, Mercer RW (1998) Isozymes of the NaK-ATPase: heterogeneity in structure, diversity in function. Am J Physiol 275:F633–F650
3. Bosher SK, Warren RL (1971) A study of the electrochemistry and osmotic relationships of the cochlear fluids in the neonatal rat at time of the development of the endocochlear potential. J Physiol (Lond) 212:739–761
4. Cate WJF ten, Curtis LM, Rarey KE (1994) NaK-ATPase α and β subunit isoform distribution in the rat cochlear and vestibular tissues. Hear Res 75:151–160
5. Cate WJF ten, Curtis LM, Rarey KE (1994) NaK-ATPase subunit isoform expression in the guinea pig endolymphatic sac. ORL J Otorhinolaryngol Relat Spec 56:257–262
6. Couloigner V, Teixeira M, Sterkers O, Ferrary E (1999) In vivo study of the electrochemical composition of luminal fluid in the guinea pig endolymphatic sac. Acta Otolaryngol (Stockh) 119:200–202
7. Crambert G, Hasler U, Beggah AT, Yu C, Modyanov NN, Horisberger JD, Lelièvres L, Geering K (2000) Transport and pharmacological properties of nine different human NaK-ATPase isozymes. J Biol Chem 275:1976–1986
8. Erichsen S, Zuo J, Curtis L, Rarey K, Hultcrantz M (1996) NaK-ATPae α- and β-isoforms in the developing cochlea of the mouse. Hear Res 100:143–149
9. Farman N (1996) NaK-pump expression and distribution in the nephron. Miner Electrolyte Metab 22:272–278
10. Ferrary E, Sterkers O (1998) Mechanism of endolymph secretion. Kidney Int 53:S98–S103
11. Geering K, Beggah A, Good P, Girardet S, Roy S, Schaer D, Jaunin P (1996) Oligomerization and maturation of NaK-ATPase: functional interaction of the cytoplasmic H-2 terminus of the β subunit with the α subunit. J Cell Biol 133:1193–1204
12. Hieber V, Siegel GJ, Fink DJ, Beaty MW, Mata M (1991) Differential distribution of NaK-ATPase alpha isoforms in the central nervous system. Cell Mol Neurobiol 11:253–262
13. Kuijpers W, Bonting SL (1970) The cochlear potentials II. The nature of the cochlear endolymphatic resting potential. Pflugers Arch 320:359–372
14. Kuijpers W (1974) NaK-ATPase activity in the cochlea of the rat during development. Acta Otolaryngol (Stockh) 78:341–344
15. Kuijpers W, Peters TA, Tonnaer ELGM, Ramaekers FCS (1991) Expression of cytokeratin polypeptides during development of the rat inner ear. Histochemistry 96:511–521
16. Lavoie L, Levenson R, Martin-Vasallo P, Klip A (1997) The molar ratios of α and β subunits of the NaK-ATPase differ in distinct subcellular membranes from rat skeletal muscle. Biochemistry 36:7726–7732
17. Magyar JP, Bartsch U, Wang ZQ, Howells N, Aguzzi A, Wagner EF, Schachner M (1994) Degeneration of neural cells in the central nervous system of mice deficient in the gene for the adhesion molecule on glia, the β2 subunit of murine NaK-ATPase. J Cell Biol 27:835–845
18. McGuirt JP, Schulte BA (1994) Distribution of immunoreactive α- and β-subunit isoforms of NaK-ATPase in the gerbil inner ear. J Histochem Cytochem 42:843–853
19. McGuirt JP, Schmiedt RA, Schulte BA (1996) NaK-ATPase and carbonic anhydrase expression in the developing gerbil cochlea. Auditory Neurosci 2:135–144
20. Ruiz A, Bhat SP, Bok D (1996) Expression and synthesis of the NaK-ATPase β2 subunit in human retinal pigment epithelium. Gene 176:237–242
21. Rybak LP, Whitworth C, Scott V (1992) Development of endocochlear potential and compound action potential in the rat. Hear Res 59:189–194
22. Schmalzing G, Ruhl K, Gloor SM (1997) Isoform-specific interactions of NaK-ATPase subunits are mediated via extracellular domains and carbohydrates. Proc Natl Acad Sci USA 94:1136–1141
23. Spicer SS, Schulte BA (1996) The fine structure of spiral ligament cells related to ion return to the stria and varies with place-frequency. Hear Res 106:80–100
24. Sweadner KJ (1989) Isozymes of the NaK-ATPase. Biochim Biophys Acta 988:185–220
25. Teixeira M, Couloigner V, Loiseau A, Hulin P, Sterkers O, Planelles G, Ferrary E (1999) Evidence for apical K^+ conductance and NaK2Cl cotransport in the endolymphatic sac of guinea pig. Hear Res 128:45–50
26. Therien AG, Blostein R (1999) K^+/Na^+ antagonism at cytoplasmic sites of NaK-ATPase: a tissue-specific mechanism of sodium pump regulation. Am J Physiol 277:C891–C898
27. Therien AG, Nestor NB, Ball WJ, Blostein R (1996) Tissue-specific versus isoform specific differences in cation activation kinetics of the NaK-ATPase. J Biol Chem 271:7104–7112
28. Tonnaer ELGM, Kuijpers W, Peters TA, Ramaekers FCS (1990) Effect of EDTA on cytokeratin detection in the inner ear. J Histochem Cytochem 38:1223–1227
29. Wangemann P (1995) Comparison of ion transport mechanisms between vestibular dark cells and strial marginal cells. Hear Res 90:149–157
30. Zlokovic BV, Mackic JB, Wang L, McComb JG, McDonough A (1993) Differential expression of NaK-ATPase α and β subunit isoforms at the blood-brain barrier and the choroid plexus. J Biol Chem 286:8019–8025
31. Zuo J, Curtis LM, Yao X, ten Cate WJF, Rarey KE (1995) Expression of NaK-ATPase α and β isoforms in the neonatal rat cochlea. Acta Otolaryngol (Stockh) 115:497–503