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Na-K-ATPase activity in the guinea pig stria vascularis in experimentally-induced endolymphatic hydrops

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Summary. The effect of endolymphatic hydrops on the Na-K-ATPase activity in the guinea pig stria vascularis was electron microscopically and enzyme cytochemically investigated one year after experimental induction. The morphological observations revealed intercellular dropsy in the basal infoldings of the marginal cells, and shrinkage and disappearance of intermediate cells. Moreover, shrinkage of the marginal cells, especially of the basal infoldings, was occasionally observed. In spite of these morphological alterations, the Na-K-ATPase activity was still detected on the plasma membrane of the basal infoldings of most marginal cells. No remarkable differences were found among the cochlear turns of the specimens examined. However, no reaction product was detected on the basolateral plasma membrane of severely degenerated marginal cells. The present results indicate that the Na-K-ATPase of the plasma membrane of the basal infoldings of the marginal cells plays an important role in the maintenance of the unique ion concentration of the endolymph even in the endolymphatic hydropic condition, and that the Na-K-ATPase activity is attenuated in severely atrophic cells.

Key words: Na-K-ATPase, Endolymphatic hydrops, Stria vascularis, Guinea pig

Introduction

The Na-K-ATPase is well known to participate in the active transport of Na^+ and K^+ ions (Skou, 1965; Whittam and Wheeler, 1970). In the stria vascularis, the enzyme activity is normally localized in the plasma membrane of the basal infoldings of the marginal cells (Seguchi and Kobayashi, 1983; Kobayashi et al., 1985, 1987), and is currently believed to regulate the high Na^+ and low K^+ ion concentration of the endolymph. Several morphological and biochemical studies on the effect of endolymphatic hydrops in the stria vascularis have been conducted so far (Kimura, 1967; Konishi and Kelsey, 1976; Cohen and Morizono, 1984; Albers et al., 1987; Ruding et al., 1987), but no ultracytochemical study of the Na-K-ATPase activity in experimentally-induced endolymphatic hydrops has been reported yet. The present study was thus undertaken to investigate the cytochemical localization of this enzyme activity in the stria vascularis of the guinea pig one year after experimental induction.

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Materials and methods

Seven female guinea pigs of Hartley strain weighing 250-350 g (Shizuoka Experimental Animal Co-op, Shizuoka, Japan) with normal Preyer's reflex were employed. Under sodium pentobarbital anaesthesia, the right endolymphatic sac and duct were surgically obliterated according to the method of Kimura and Schuknecht (1965).

One year after operation, the animals were perfused through the left ventricle, under sodium pentobarbital anaesthesia, with a fixative containing 2% paraformaldehyde and 0.05% glutaraldehyde in 0.1M cacodylate buffer, pH 7.4, for 10 min at 4 °C. After decapitation the whole right cochlea was quickly removed from the temporal bone, and the bony capsule was carefully chipped off under a stereomicroscope while immersed in the same fixative as above. Swelling of the Reissner's membrane toward the scala vestibuli was examined to confirm the existence of endolymphatic hydrops. The unoperated specimens from the left ear were used as control. The stria vascularis with the spiral ligament were dissected out from the excised cochlear duct and immersed in the same fixative for 1 hr at 4 °C.

After fixation, the specimens were washed overnight in 0.1M cacodylate buffer, pH 7.4, at 4 °C. The incubation was performed in the reaction medium of Kobayashi et al. (1987). Control experiments included replacement of the K^+ ions in the medium for Na^+ ions, addition of 10 mM ouabain, and omission of the

substrate. When the reaction was completed, the tissue was postfixed in 1% OsO_4, dehydrated through a series of graded ethanols and embedded in Spurr's epoxy resin (Spurr, 1969). Uncontrasted ultrathin sections were observed under a JEM-100S electron microscope (JEOL Co., Tokyo, Japan).

The experiment above was performed in accordance with the guidelines of the Declaration of Helsinki.

Results

The reaction product of the Na-K-ATPase activity in the stria vascularis of the control specimens was found along the plasma membrane of the basal infoldings of the marginal cells. No reaction was detected in either the intermediate or the basal cells.

The morphological alterations observed on the specimens with endolymphatic hydrops included dropsy at the intercellular spaces between the basal infoldings of the marginal cells (Fig. 1), shrinkage (Fig. 2) and disappearance of intermediate cells, vacuolation, and areas where marginal cells were in the process of shrinking (Figs. 3, 4). Shrinkage of the marginal cells was particularly evident in the basal infoldings. No remarkable morphological changes were observed in the basal cells.

In the stria vascularis of the hydropic right side cochlea, positive reaction of the Na-K-ATPase activity was still observed at the plasma membrane of the basal infoldings of the marginal cells (Figs. 1, 2) except for some portions undergoing marked shrinkage (Figs. 3, 4). All the cochlear turns were examined in 4 of the 7 guinea pigs employed, but no clear difference was seen as regards the reaction pattern or the morphological changes.

In the specimens incubated in the different control media, the reaction product of the Na-K-ATPase activity had either almost disappeared or was not detected at all.

Discussion

The reaction detected in the striae vasculares of the control specimens from the left ear was restricted to the plasma membrane of the basal infoldings of the marginal cells, which coincided with the findings previously reported (Kobayashi et al., 1987).

The ultrastructural changes brought about by endolymphatic hydrops in the stria vascularis of the guinea pig have been previously studied in specimens taken at relatively early stages after the experimental induction (Kimura, 1967; Albers et al., 1987; Ruding et al., 1987). According to Albers et al. (1987), intercellular dropsy appears one month after treatment, and shrinkage of the intermediate cells at three months. In the present study, similar morphological changes were observed one year after the experimental induction of endolymphatic hydrops (Figs. 1, 2). In some areas, these changes had further progressed: intermediate cells were deformed and finally disappeared, probably leading to the vacuolation observed in the stria vascularis. In the areas displaying changes of such magnitude, the marginal cells, unable to

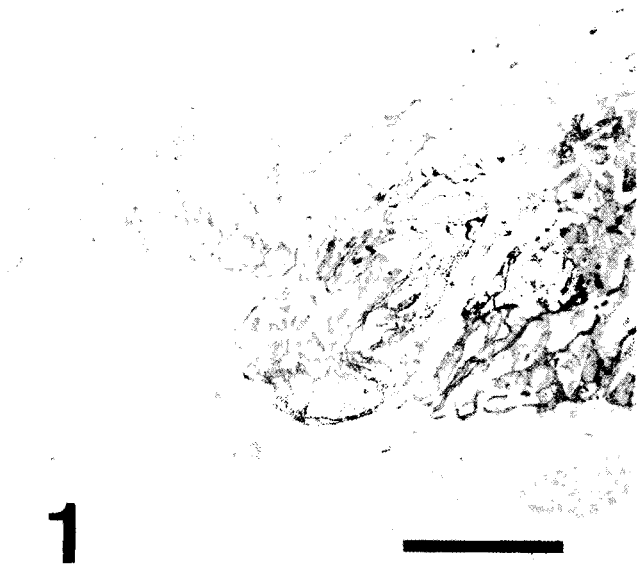

Fig. 1. Dropsy is observed at the intercellular space between the basal infoldings of the marginal cells. The Na-K-ATPase activity is detected on the plasma membrane of the basal infoldings of the marginal cells. Bar= 4 μm. x 5,300

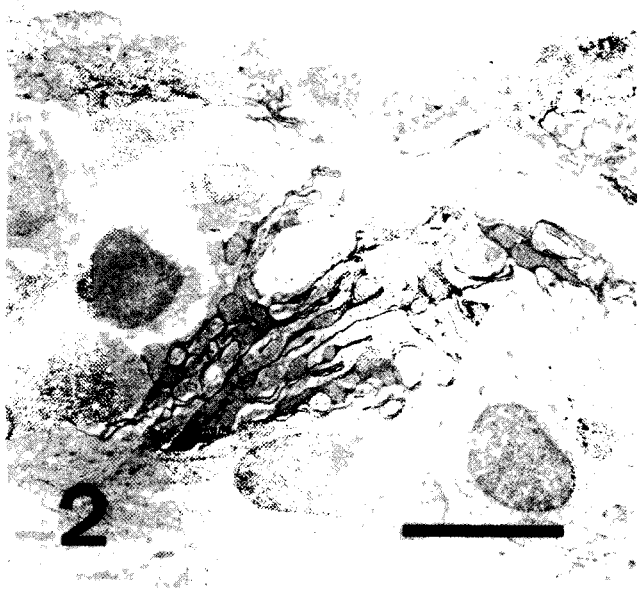

Fig. 2. In addition to dropsy at the intercellular space between the basal infolding of the marginal cells, shrinkage of the intermediate cells is observed. The Na-K-ATPase activity is localized on the plasma membrane of the basal infoldings of the marginal cells. Bar= 4 μm. x 5,300

maintain the normal structure of their basal infoldings, shrank (Figs. 3, 4). If the endolymphatic hydropic condition had been allowed to continue the degree of shrinkage of the marginal cells would have probably become higher, and the number of collapsed marginal cells would have increased.

The Na^+ and K^+ ion concentration of the endolymph in guinea pigs under experimentally-induced endolymphatic hydrops has been reported to be similar to that of normal animals (Konishi and Kelsey, 1976; Cohen and Morizono, 1984). The fact that, in spite of the morphological changes observed in the stria vascularis, the Na-K-ATPase activity was detected in the basal infoldings of most of the marginal cells one year after the experimental induction of endolymphatic hydrops indicates that, in this area, the Na^+ and K^+ ionic

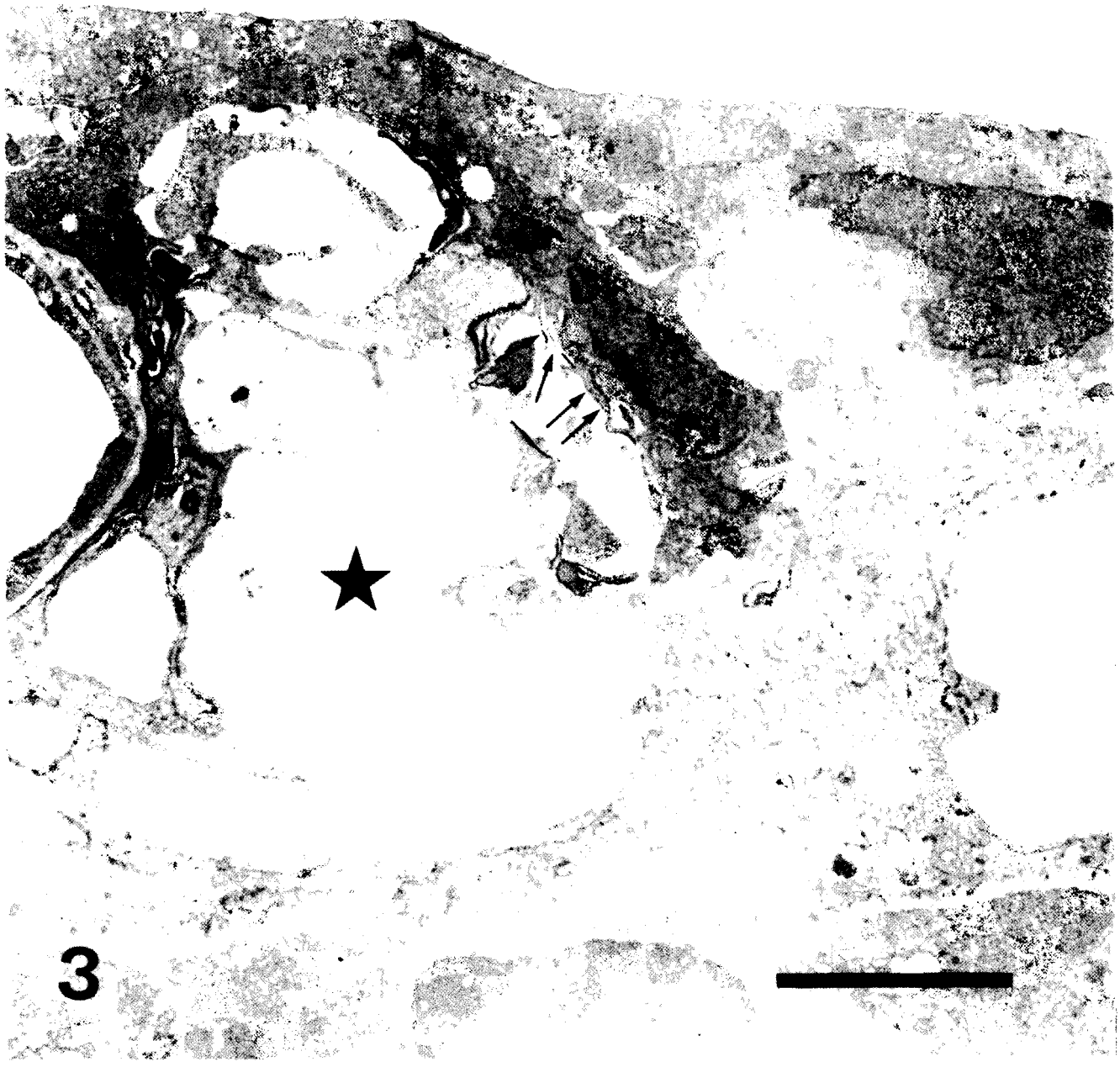

Fig. 3. Intermediate cells about to collapse (star). The marginal cells around them are shrunken too. The Na-K-ATPase activity is not detected in the shrunken basal plasma membrane of the marginal cells (arrows). Bar= 3 μm. x 12,400

exchange is still being performed, and that even under endolymphatic hydropic conditions it plays an important role in the control of the Na^+ and K^+ ion concentration of the endolymph.

Since the shrinkage in the marginal cells occurs mainly in the basal infoldings, and specially since the cytoplasm of some of these cells was negative for the enzyme activity, it is reasonable to think that if the condition progressed, the total Na-K-ATPase activity of the stria vascularis would be markedly decreased. It is, therefore, our intention to pursue the study of endolymphatic hydrops in specimens taken from animals subjected to this condition for longer periods of time.

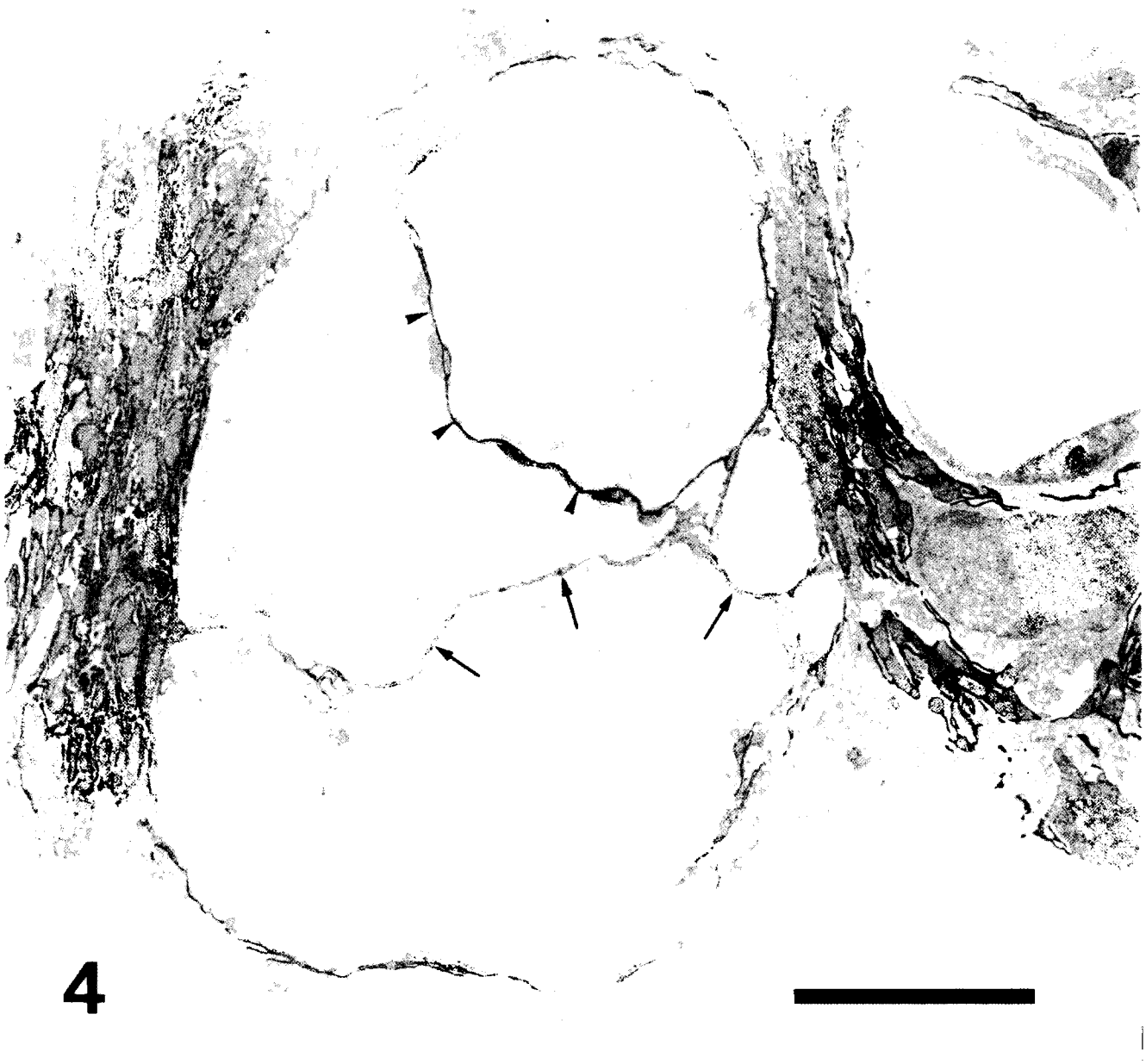

Fig. 4. Vacuolation, due to shrinkage of the intermediate cells, is observed. Under this condition, processes of the marginal cells are seen dangling. The localization of the Na-K-ATPase activity varies from place to place (arrow: negative reaction, arrowhead: positive reaction). Bar= 7 µm. x 5,300

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