Histol Histopathol (2001) 16: 1197-1201

http://www.ehu.es/histol-histopathol

Histology and Histopathology

Cellular and Molecular Biology

Review

Cytochemical localization of Na^+/K^+-ATPase activity in cochlear strial marginal cells after various catecholamine administrations

N. Kanoh[1], C.-F. Dai[2], D. Mohri[1] and S. Hori[3]

[1]Departments of Otolaryngology, and [3]Physiology, Hyogo College of Medicine, Nishiomiya, Hyogo, Japan and

[2]Department of Otolaryngology, Eye Ear Nose and Throat Hospital, Shanghai Medical Univeristy, Shanghai, P.R. China

Summary. Sodium/potassium-activated adenosine triphosphatase (Na^+/K^+-ATPase) activity in the kidney and brain is high, and is regulated by catecholamines. Na^+/K^+-ATPase activity is also high in the basolateral infoldings of the strial marginal cells, where it aids in maintaining the characteristic electrolyte composition of the endolymph. To clarify the involvement of humoral control in strial function, particularly the role of catecholamines, the K^+-dependent *p*-nitrophenyl-phosphatase (K^+-NPPase) activity of strial marginal cells was investigated in guinea pigs using a cerium-based cytochemical method. The effects of reserpine, serotonin (5-HT), norepinephrine (NE), epinephrine (EP), both alone and in combination, were studied. High doses of reserpine cause depletion of sympathetic substances. Strial K^+-NPPase activity was decreased after reserpine or dopamine treatment, and was increased after 5-HT, NE, and EP treatment. After reserpinization, repeated treatment with 5-HT, NE, or EP led to detectable strial enzyme activity. Thus, exogenous 5-HT, NE, and EP were able to restore strial K^+-NPPase activity in the reserpine-treated animals. These results suggested that biogenic amines regulate strial K^+-NPPase activity. Thus, the function of the stria vascularis may be regulated by the opposing actions of these catecholamines, and 5-HT.

Key words: Na^+/K^+-ATPase, Stria vascularis, Catecholamine, Biogenic amine, Humoral control, Cytochemistry

Introduction

Sodium/potassium-activated adenosine triphosphatase (Na^+/K^+-ATPase) is a highly conserved membrane enzyme that is essential for cellular homeostasis (Skou, 1965; Whittam and Wheeler, 1970; Jorgensen, 1980). This enzyme plays a "housekeeping" role by maintaining low Na^+ and high K^+ concentrations in the intracellular milieu, which are crucial for preserving the membrane potential of all cells (Ewart and Klip, 1995). In strial marginal cells, Na^+/K^+-ATPase activity is high and contributes to the characteristic electrolyte composition of the endolymph (Smith et al., 1954; Stecker et al., 1988; Kanoh and Makimoto, 1984, 1985). Cochlear endolymph contains high levels of K^+ and low levels of Na^+, thus resembling intracellular fluid. However, the stria vascularis is reported not to be innervated (Terayama et al., 1966; Spoendlin and Lichtensteiger, 1966; Shibamori et al., 1994). Therefore, elucidating of the mechanisms by which the stria vascularis is regulated is now an important goal.

In 1995, Ewart and Klip reviewed the hormonal regulation of Na^+/K^+-ATPase in various tissues, stating that this enzyme is under both short- and long-term control by a number of circulating hormones such as aldosterone, thyroid hormones, and catecholamines. The long-term regulation exerted by thyroid hormone and aldosterone is mediated by changes in gene expression. The short-term regulation exerted by catecholamines is mediated by reversible phosphorylation of the catalytic subunit of the Na^+/K^+-ATPase. In 1992, Hernandez reviewed the effects of extrinsic catecholamines and serotonin (5-HT) on Na^+/K^+-ATPase activity in the brain. Norepinephrine (NE) enhances and stimulates Na^+/K^+-ATPase activity in the brain (Clausen and Formby, 1967) and in rat brown adipose tissue membranes (Herd et al., 1970). In the cerebral cortex, 5-HT increases enzyme activity (Hernandez, 1979). In the kidney, NE stimulates Na^+/K^+- ATPase activity and decreases urinary sodium excretion (Sundaresan et al., 1987). However, dopamine and dopamine agonists inhibit Na^+/K^+-ATPase activity in the proximal tubules (Aperia et al., 1987), in the thick ascending limb of Henle's loop (Meister et al., 1989), and in the cortical collecting tubules (Seri et al., 1988), thus leading to increased natriuresis (Meister and Aperia, 1993). These

Offprint requests to: Naoyuki Kanoh, MD, Department of Otolaryngology, Hyogo College of Medicine, 1-1 Mukogawacho, Nishinomiya, Hyogo 663-8501, Japan. e-mail: nkanoh@hyo-med.ac.jp

catecholamines have pronounced effects on the renal handling of sodium and water, with NE promoting sodium and water retention, and dopamine-promoting sodium and water excretion (Ibarra et al., 1993).

Strial K^+-NPPase in the cochlea (Table 1)

Ouabain-sensitive, K^+-dependent *p*-nitrophenyl-phosphatase (K^+-NPPase) is the second component of the Na^+/K^+-ATPase complex, which represents the dephosphorylation step in the sodium pump cycle (Judah et al., 1962). Using a cerium-based method (Kobayashi et al., 1987), K^+-NPPase activity was localized to the basolateral infoldings of cochlear strial marginal cells (Fig. 1), but enzyme activity was decreased from day 3 to day 20 after reserpine treatment (Fig. 2; Kanoh et al., 1993; Kanoh, 1994). A similar decrease was observed for the facial nerve (Kanoh, 1997), choroid plexus (Dai and Kanoh, 1998), and kidney (unpublished data). After administering biogenic amines, such as EP (Kanoh, 1999), NE (Kanoh, 1998a), or 5-HT (Kanoh et al. 1998), strial K^+-NPPase activity was detectable at a level similar to that of normal untreated animals. However, after dopamine treatment, strial K^+-NPPase activity was undetectable (Fig. 3; Kanoh, 1995). A similar decrease was found for choroid plexus (Kanoh, 1998b). Hence, administration of EP (Fig. 4; Kanoh, 1999), NE (Fig. 5; Kanoh, 1998a), or 5-HT (Fig. 6; Kanoh et al., 1998) after reserpine treatment restores strial K^+-NPPase activity.

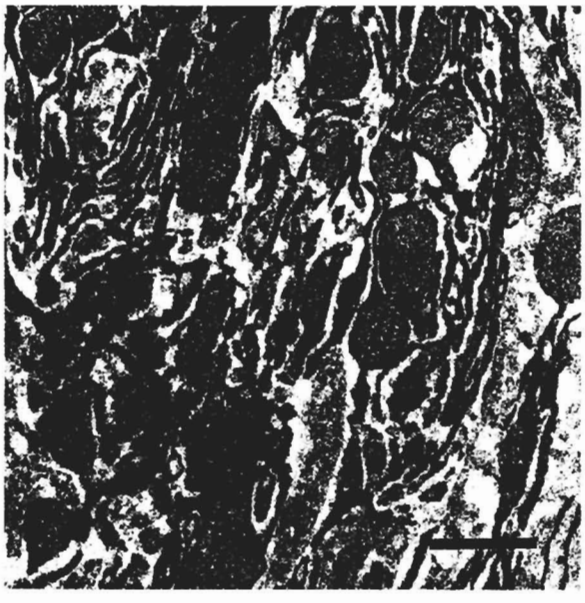
For controls of negative enzyme reactivity, strial K^+-NPPase activity was investigated after incubation with 10 mM ouabain, substrate free medium, and K^+ was replaced with Na^+ from the incubation medium.

Table 1. Strial K^+-NPPase activity after biogenic amine administrations.

ENZYME ACTIVITY	RESERPINE	EP	NE	DOPAMINE	5-HT
Brain	--	++	++		
Kidney	--	++	++	--	
Brown adipose tissue			++		
Choroid plexus	--	++	++		
Stria vascularis (present study)	--	++	++	--	++

Discussion

Ouabain-sensitive, K^+-dependent, Na^+/K^+-ATPase participates in the active transport of Na^+ and K^+ ions involved in ionic and fluid homeostases throughout the body (Albers et al., 1989). For determination of the localization of Na^+/K^+- ATPase, a number of histochemical studies have been performed. Nakai and Hilding (1966) and Nomura et al. (1970) documented a positive reaction on the endolymphatic cell surface and in the intercellular spaces between the marginal and intermediate or basal cells by the modified method of Wachstein and Meisel (1957) using adenosine triphosphate (ATP) as a substrate. However, because of the non-enzymatic, lead-catalyzed hydrolysis of ATP in the reaction conditions and the existence of nucleotides in the reaction product, their results did not reflect the exact localization of Na^+/K^+-ATPase activity. Mees (1983) was the first to successfully demonstrate K^+-NPPase activity (Judah et al., 1962) in the stria vascularis by the strontium method of Ernst (1972a,b). Kobayashi et al. (1985) also obtained the same results by



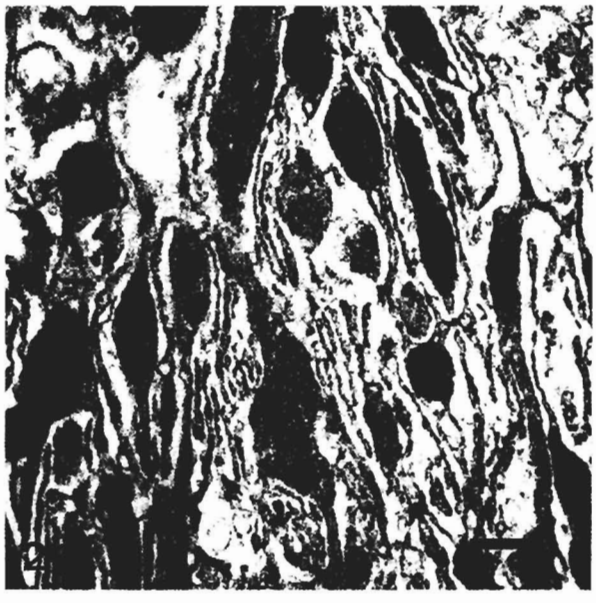


Fig. 1. Normal animal. The fine reaction product of K^+-NPPase activity is localized to the cytoplasmic side of the basolateral infoldings of cochlear strial marginal cells. Bar: 1 μm.

Fig. 2. Reserpinized animal. Reaction product is almost undetectable. Bar: 1 μm.

the lead-based method of Mayahara et al. (1980), and recently they confirmed their data by their own cerium-based method (1987). Thus, the localization of K^+-NPPase activity was established to the plasma membrane of the basolateral infoldings of the marginal cells. Our findings in untreated normal animals were consistent with these previous reports.

The cytochemical methods can be used to determine positive or negative K^+-NPPase reactivity. If the enzyme reaction product is undetectable, then K^+-NPPase reactivity is extremely low. If the K^+-NPPase reactivity is markedly increased, then the reaction product will be detectable, as in the normal state. However, cytochemistry is said not to be useful for detecting

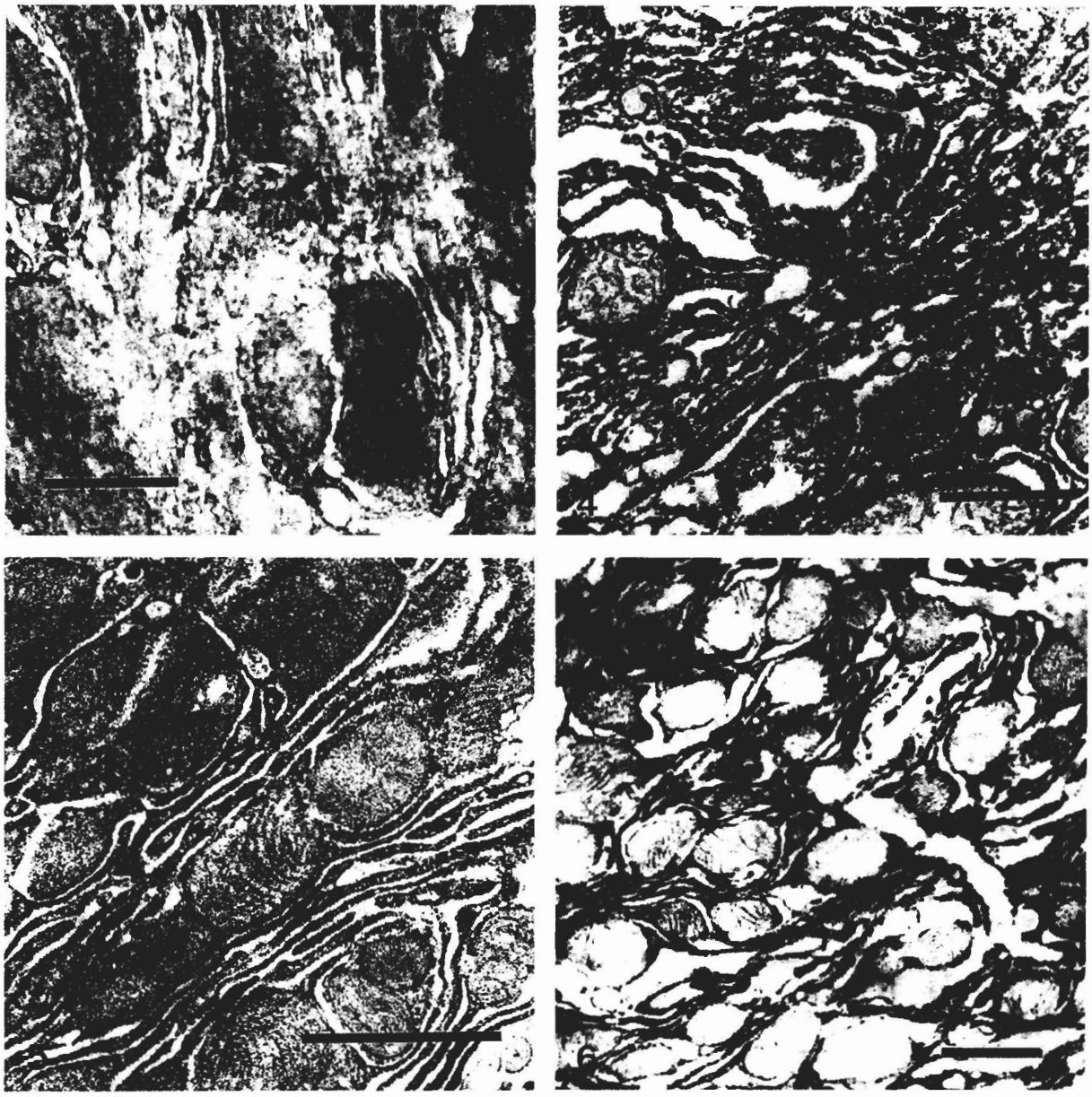


Fig. 3. Dopamine treatment. Reaction product is unobserved. Bar: 1 μm.

Fig. 4. EP treatment after reserpinization. Reaction product is detectable. Bar: 1 μm

Fig. 5. NE treatment after reserpinization. Reaction product is observed. Bar: 1 μm.

Fig. 6. 5-HT after reserpinization. Reaction product is distributed in strial marginal cells. Bar: 1 μm.

increases in K^+-NPPase activity. This study developed a two-step cytochemical procedure for detecting increases in K^+-NPPase activity. In the first step, the target agents (catecholamines and 5-HT) are administered alone and the enzyme activity is determined. When enzyme reactivity is proven positive, the second step is performed; the target agents (catecholamines and 5-HT) are administered following reserpinization. Enzyme activity is again examined. If the reactivity is now positive, then these target agents must have increased the enzyme reactivity.

Pharmacologically, reserpine releases biological amines such as NE, EP, dopamine, and 5-HT from storage or binding sites in the central and peripheral nervous system. Thus, high doses of reserpine induce the depletion of these amines, inhibit their reabsorption at the storage sites, and prevent their reassociation at the binding sites. In our previous studies, transporting Na^+/K^+-ATPase activity on the internodal axolemma of the guinea pig facial nerve was found to be decreased after reserpinization (Kanoh, 1997), the K^+ concentration of the cochlear endolymph was found to be decreased 24 h after reserpine administration (Kanoh and Makimoto, 1984), and strial K^+-NPPase activity was almost completely attenuated from day 3 to day 20 after reserpinization (Kanoh et al., 1993; Kanoh, 1994). The dose of reserpine used (10 mg/kg) was thought to be sufficient to completely abolish catecholamine activity (Wakade, 1980). These experiments were performed with 17 guinea pigs to allow for an optimal experimental protocol, six animals subsequently died. Clinically, reserpine is employed for the treatment of hypertension. The dosage employed in the present experiments is 500 to 1000 times higher than the clinical dosage. In a pilot study of reserpinized animals, the systolic and diastolic blood pressures in the femoral artery decreased to 66.6% and 75.1% of normal levels, respectively, one day after reserpine administration. These values rose to essential normal levels by day seven. Therefore, day 10 after reserpine administration represents a reasonable checkpoint for evaluating the effects of reserpine and catecholamines on K^+-NPPase activity.

The purpose of this study was to evaluate the cytochemical effects of catecholamine depletion on strial Na^+/K^+-ATPase activity, and to clarify the relationship between strial K^+-NPPase activity and exogenous catecholamines as well as 5-HT. Table 1 summarizes previous reports on the administration of various biogenic amines and our data regarding the effects of catecholamines and 5-HT administration following reserpinization on strial K^+-NPPase activity. Strial K^+-NPPase activity after five repeated doses of NE or 5-HT was found to be almost positive. L-threo-DOPS (L-threo-3,4-dihydroxyphenylserine), an L-NE precursor (Sasa et al., 1987), also showed positive reactivity for K^+-NPPase (Kanoh and Nomura, 1995). Thus, the observations of NE treatment support the results on DOPS. However, a high dose of dopamine hydrochloride was found to induce negative enzyme reactivity (Kanoh, 1995). NE, DOPS, or 5-HT administration after reserpinization led to positive strial K^+-NPPase activity, showing that these agents were able to restore activity. Although the subunits of strial K^+-NPPase are different from those in the kidney and brain (Shyjan and Levenson., 1989; Ryan and Watts, 1991; Watts et al., 1991), our findings indicate that NE and 5-HT increased strial Na^+/K^+-ATPase activity, and that dopamine decreases Na^+/K^+-ATPase activity, as in the kidney and brain. In the near future, these findings will be clinically useful for investigating the pathogenesis of endolymphatic hydrops in Meniere's disease.

Acknowledgements. The authors thank Prof. Harumichi Seguchi, and Associate Prof. Teruhiko Okada, Department of Anatomy and Cell Biology, Kochi Medical School, and Prof. Masafumi Sakagami, Department of Otolaryngology, Hyogo College of Medicine, for their valuable comments. This study was supported by a Grant-in-Aid (08571998, Kanoh) for Science Research from the Ministry of Education, Science and Culture of Japan.

References

Albers R.W., Siegel, G.J. and Stahl W.C. (1989). Membrane transport. In: Basic neurochemistry. Siegel G.J., Agrranoff B.W., Albers R.W. and Molinoff P.B. (eds). Raven Press. New York. pp 49-70.

Aperia A., Bertorello A. and Seri I. (1987). Dopamine causes inhibition of Na^+, K^+-ATPase activity in rat proximal convoluted tubule segments. Am. J. Physiol. 252, F 39-45.

Clausen J. and Formby B. (1967). Effect of noradrenaline on phosphate activity in synaptic membrane of the rat brain. Nature 213, 389-390.

Dai C.F. and Kanoh N. (1998). Cytochemical localization of ouabain-sensitive K^+-dependent *p*-nitrophenylphosphatase activity in the choroid plexus in of normal and reserpinized guinea pigs. J. Histochem. Cytochem. 46, 975-976.

Ernst S.A. (1972a). Transport adenosine triphosphatase cytochemistry. I. Biochemical characterization of a cytochemical medium for the ultrastructural localization of ouabain-sensitive, potassium-dependent phosphatase activity in the avian salt gland. J. Histochem. Cytochem. 20, 13-22.

Ernst S.A. (1972b). Transport adenosine triphosphatase cytochemistry. II. Cytochemical localization of ouabain-sensitive, potassium-dependent phosphatase activity in the secretory epithelium of the avian salt gland. J. Histochem. Cytochem. 20, 23-38.

Ewart H.S. and Klip A. (1995). Hormonal regulation of the Na^+-K^+-ATPase: mechanisms underlying rapid and sustained changes in pump activity. Am. J. Physiol. 269, C 295-311.

Herd P.A., Horwitz B.A. and Smith R.E. (1970). Norepinephrine-sensitive Na^+/K^+-ATPase activity in brown adipose tissue. Experientia 26, 825-826.

Hernandez R.J. (1979). Na^+-K^+-ATPase activity in the brain cortex of rats ontogenetically malnourished, and treated with serotonin precursors. Brain Res. 162, 348-358.

Hernandez R.J. (1992). Na^+/K^+-ATPase regulation by neurotransmitters. Neurochem. Int. 20, 1-10.

Ibarra F., Aperia A., Svensson L.-B., Eklof A.-C. and Greengard P. (1993). Bidirectional regulation of Na^+,K^+-ATPase activity by dopamine and an α-adrenergic agonist. Proc. Natl. Acad. Sci. USA

90, 21-24.

Jorgensen P.L. (1980). Sodium and potassium ion pump in Kidney tubules. Physiol. Rev. 60, 864-917.

Judah J.D., Ahmed K. and McLean A.E.M. (1962). Ion transport and phosphoproteins of human red cell. Biochem. Biophys. Acta 65, 472-484.

Kanoh N. (1994). Reserpine inhibits the Na-K ATPase activity of the stria vascularis in the cochlea. Laryngoscope 104, 197-200.

Kanoh N. (1995). Dopamine inhibits the Na-K ATPase activity of the stria vascularis in the cochlea. Acta Otolaryngol. (Stockh.) 115, 27-30.

Kanoh N. (1997). Cytochemical localization of ouabain-sensitive K^+-dependent *p*-nitrophenylphosphatase activity in the facial nerve of reserpinized guinea pigs. J. Histochem. Cytochem. 45, 1129-1135.

Kanoh N. (1998a). Effect of norepinephrine on ouabain-sensitive K^+-dependent *p*-nitrophenylphosphatase activity in strial marginal cells of the cochlea in normal and reserpinized guinea pigs. Acta Otolaryngol. 118, 817-820.

Kanoh N. (1998b). Effects of dopamine hydrochloride (Inovan") on ouabain-sensitive K^+-dependent *p*-nitrophenylphosphatase activity of choroid plexus in guinea pigs. Brain Res. 787, 154-156.

Kanoh N. (1999). Effects of epinephrine on ouabain-sensitive K^+-dependent *p*-nitrophenylphosphatase activity in strial marginal cells of guinea pigs. Ann. Otol. Rhinol. Laryngol. 108, 345-348.

Kanoh N. and Makimoto K. (1984). Effects of reserpinization on the electrolytes. Distribution in inner ear fluids of guinea pig. Acta Otolaryngol. (Stockh.) 98, 98-104.

Kanoh N. and Makimoto K. (1985). The effects of peroral glycerol administration on inner ear fluid electrolytes. Ann. Otol. Rhinol. Laryngol. 94, 319-321.

Kanoh N. and Nomura J. (1995). The role of L-threo-DOPS in the control of Na-K ATPase activity of the marginal cells in the stria vascularis of reserpinized guinea pigs. Acta Otolaryngol. (Stockh.) Suppl. 520, 381-383.

Kanoh N., Kumoi T., Okada T. and Seguchi H. (1993). Ultracytochemical study of ouabain-sensitive K^+-dependent p-nitrophenylphosphatase activity in the stria vascularis of reserpinized guinea pigs. Acta Otolaryngol. (Stockh.) 113, 142-145.

Kanoh N., Hori K., Ishigaki T. and Hori S. (1998). Effects of serotonin on ouabain-sensitive K^+-dependent p-nitrophenylphosphatase activity in strial marginal cells of normal and reserpinized guinea pigs. Histochem. J. 30, 263-266.

Kobayashi T., Seguchi H., Okada T. and Yagyu K. (1985). Ultracytochemical study of the stria vascularis of the guinea pig cochlea. Anat. Anz. 160, 101-114.

Kobayashi T., Okada T. and Seguchi H. (1987). Cerium-based cytochemical method for detection of ouabain-sensitive, potassium-dependent, *p*-nitrophenylphosphatase activity at physiological pH. J. Histochem. Cytochem. 35, 601-611.

Mayahara H., Fujimoto K., Ando T. and Ogawa K. (1980). A new one-step method for the cytochemical localization of ouabain-sensitive, potassium-dependent *p*-nitrophenylphosphatase activity. Histochemistry 67, 125-138.

Mees K. (1983). Ultrastructural localization of K^+-dependent, ouabain-sensitive NPPase (Na-K ATPase) in the guinea pig inner ear. Acta Otolaryngol. (Stockh.) 95, 277-289.

Meister B. and Aperia A. (1993). Molecular mechanisms involved in catecholamine regulation of sodium transport. Semin. Nephrol. 13, 41-49.

Meister B., Fryckstedt J., Schalling M., Cortes R., Hokfelt T., Hemmings H.C., Nairn A.C., Ehrlich M. and Greengard P. (1989). Dopamine- and cAMP-regulated phosphoprotein (DARPP-32) and DA1-agonist-sensitive Na^+, K^+-ATPase in tubulecells of the kidney. Proc. Natl. Acad. Sci. USA 86, 8068-8072.

Nakai Y. and Hilding D.A. (1966). Electron microscopic studies of adenosine triphosphatase activity in the stria vascularis and spiral ligament. Acta Otolaryngol. (Stockh.) 62, 411-428.

Nomura Y., Ishii T. and Ishii D. (1970). The histochemistry of the spiral prominence. Arch. Klin. Exp. Ohren-Nasen-Kehlkopfheilkd (Berlin) 195, 266-275.

Ryan A.F. and Watts A.G. (1991). Expression of mRNAs encoding α and ß subunit isoforms of the Na,K-ATPase in the rat cochlea. Mol. Cell Neurosci. 2, 179-187.

Sasa M., Ohno Y., Nabatame H., Yoshimura N. and Takaori S. (1987). Effects of L-threo-DOPS, an L-noradrenaline precursor, on locus coeruleus-originating neurons in spinal trigeminal nucleus. Brain Res. 420, 157-161.

Seri I., Kone B.C., Gullans S.R. and Aperia A. (1988). Locally formed dopamine inhibits Na^+-K^+-ATPase activity in rat renal cortical tubule cells. Am. J. Physiol. 255, F 666-673.

Shibamori Y., Tamamaki N., Saito H. and Nojyo Y. (1994). The trajectory of the sympathetic nerve fibers to the rat cochlea as revealed by anterograde and retrograde WGGA-HRP tracing. Brain Res. 646, 223-229.

Shyjan A.W. and Levenson R. (1989). Antisera specific for the α1, α2, α3, and ß subunits of the Na,K-ATPase: differential expression of α and ß subunits in rat tissue membranes. Biochemistry 28, 4531-4535.

Skou J.C. (1965). Enzymatic basis for active transport of Na^+ and K^+ across cell membrane. Physiol. Rev. 45, 596-617.

Smith C.A., Lowry O.H. and Wu M.L. (1954). Electrolytes of labyrinthine fluids. Laryngoscope 64, 141-153.

Spoendlin H. and Lichtensteiger W. (1966). The adrenergic innervation of the labyrinth. Acta Otolaryngol. (Stockh.) 61, 423-434.

Sterkers O., Ferrary E. and Amiel C. (1988). Production of inner ear fluids. Physiol. Rev. 68,1083-1128.

Sundaresan P.R., Guarnaccia M.M. and Izzo J.L. Jr. (1987). Adrenal medullary regulation of rat renal cortical adrenergic receptors. Am. J. Physiol. 253, F 1063-1067.

Terayama Y., Holz E. and Beck C. (1966). Adrenergic innervation of the cochlea. Ann. Otol. Rhinol. Laryngol. 75, 69-86.

Wachstein M. and Meisel E. (1957). Histochemistry of hepatic phosphatase at a physiological pH. Am. J. Clin. Pathol. 27, 13-23.

Wakade A.R. (1980). A comparison of rates of depletion and recovery of noradrenaline sores of peripheral and central noradrenergic neurons after reserpine administration. Importance of neuronal activity. Br. J. Pharmacol. 68, 93-98.

Watts A.G., Sanchez-Watts G., Emanuel J.R. and Levenson R. (1991). Cell-specific expression of mRNAs encoding Na^+,$K(^+)$-ATPase alpha- and beta-subunit isoforms within the rat central nervous system. Proc. Natl. Acad. Sci. USA. 88, 7425-7429.

Whittam R. and Wheeler K.P. (1970). Transport across cell membranes. Annu. Rev. Physiol. 32, 21-60.

Accepted May 25, 2001