

Increased antisecretory factor reduces vertigo in patients with Ménière's disease: a pilot study

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Received 8 August 2003; received in revised form 30 October 2003; accepted 30 October 2003

Abstract

It has been hypothesized that the symptoms of vertigo in patients with Ménière's disease somehow are related to impaired production and/or transport of endolymph. Antisecretory factor (AF) is a protein known to affect transport processes in the intestine and it has been shown that intake of specially processed cereals (SPC) can increase endogenous AF synthesis. In a prospective open pilot study, 24 patients with severe Ménière's disease (functional level scale 5–6 according to the criteria of AAO-HNS) received SPC for 14–30 days. AF levels in plasma increased by 83% in 20 of the 24 patients studied. The attacks of rotatory vertigo were reduced, to final AAO-HNS functional level scale 1–3, in 12 patients and in three of these hearing was normalized. Twelve patients had no or minor effects of the treatment. The correlation between AF activity after treatment and the final AAO-HNS functional level scale was -0.65 , $P < 0.001$. Studies in rats using immunohistochemistry methods showed that AF was localized to the cochlea and the vestibule of the inner ear. The present results suggest that AF might be a new regulator of the endolymph.

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Key words: Antisecretory factor; Ménière's disease; Immunohistochemistry

1. Introduction

Ménière's disease is characterized by recurrent episodes of severe rotatory vertigo associated with sensorineural hearing loss, tinnitus and a feeling of fullness in the affected ear. The cause is not known, but it has been hypothesized that endolymphatic hydrops that causes dilatation of the cochlear duct is involved in creating the symptoms of Ménière's disease (Hallpike and Cairns, 1938; Yamakawa, 1938; Ikeda and Sando, 1984).

Several studies have focused on the function of the endolymphatic duct and sac in the pathogenesis of the

hydrops, and histopathological evidence suggestive of decreased absorptive capacity of the endolymph has previously been demonstrated in these tissues (Rask-Andersen et al., 1981; Bagger-Sjöbäck and Rask-Andersen, 1986). Other investigators have presented evidence that endolymphatic duct and sac dysfunction cannot be considered to be the sole cause of hydrops. Furthermore, endolymphatic hydrops alone is insufficient to explain the pathophysiology of both cochlear and vestibular symptoms (Salt et al., 1986; Salt and Thalmann, 1988; Sterkers et al., 1988). Dysfunction of many structures of the inner ear including the stria vascularis, the hair cells and neurons seems increasingly likely to be involved in creating the symptoms of this disease (Kimura et al., 1986; Nadol et al., 1995). Involvement of the sympathetic nervous systems has also been indicated.

Many treatment modalities have been described for the cure of Ménière's disease. However, none are effective.

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Abbreviations: AF, antisecretory factor; SPC, specially processed cereals

tive for all patients. Treatment has focused on hydrops reduction by means of salt restriction, diuretic therapy, and various forms of adrenergic β receptor blockers have been tried. Surgical opening of the endolymphatic sac and selective resection of the vestibular nerve has also been described as being beneficial therapies for Ménière's disease. Treatment with ototoxic drugs is used to destroy a presumed defective vestibular organ.

This means that the pathophysiology of Ménière's disease is poorly understood and each of the treatments that have been described is only effective in some patients.

In the present study we have assumed that impaired production or resorption of endolymph and/or transport across membranes is involved in creating at least some of the symptoms and we tested the effect of anti-secretory factor (AF) for its potential in reducing the symptoms in patients with Ménière's disease.

AF is a 41 kDa protein originally isolated due to its ability to inhibit experimental diarrhea (Johansson et al., 1995; Lange and Lönnroth, 2001). AF probably acts as a modulator of water and ion transport. Endogenous AF synthesis can be stimulated by dietary modifications in animals and in man (Göransson et al., 1995; Björck et al., 2000). It has been reported that patients with long-standing symptoms of inflammatory bowel disease who received specially processed cereals (SPC), optimized for increasing endogenous AF synthe-

sis, had significantly increased plasma AF activity and improved subjective rating of clinical symptoms when compared to the group of patients treated with placebo cereals (Björck et al., 2000).

The aim of the present investigation was to evaluate if intake of SPC that increased AF activity could reduce the symptoms of patients suffering from Ménière's disease. We also studied the immunoreactivity of AF in a histological study in the rat inner ear.

2. Materials and methods

2.1. Patients

Twenty-four patients with 'definite Ménière's disease' according to the guidelines for the diagnosis of Ménière's disease presented by the [Committee on Hearing and Equilibrium of the American Academy of Otolaryngology - Head and Neck Surgery \(AAO-HNS\) \(1995\)](#) were studied individually over a period of 14–30 days in an open pilot study. The selected patients had severely incapacitating Ménière's disease and suffered at least two attacks weekly. The demographic data are shown in [Table 1](#).

Twelve women and 12 men, mean age 50 years (range 25–86 years) were included in the study. The right ear was affected in 13 patients and the left ear in eight

Table 1
Patient data, AF activity before and after SPC treatment, and clinical effects on AAO-HNS diagnostic staging

Patient	Sex	Age	Disease duration (years)	Side	Days of SPC treatment	AF activity		AAO-HNS ranking	
						before	after	before	after
1	F	50	(30), 0.2	(L),R	14	0.4	1.2	5	1
2	M	56	6	L	16	0.3	1.4	5	4
3	M	66	2	L	13	0.4	1.5	6	4
4	F	45	3	R	14	0.3	0.6	6	4
5	M	33	9	R	27	0.1	0.5	6	6
6	M	41	22	L	31	0	0.6	6	6
7	F	63	5	R	19	0.2	1.3	6	1
8	M	52	0.8	R	15	0	1.2	6	2
9	F	25	1	R	17	0.6	1.7	6	1
10	F	43	9	R	13	0	1.5	6	4
11	F	33	7	R	14	0	0	6	6
12	F	70	1	L	14	0	0.6	6	6
13	F	33	7	L	23	0	1.4	6	1
14	M	64	18	L	30	0.6	1.1	6	3
15	F	43	1	L	21	0.6	1.4	6	2
16	F	52	2	R	14	ND	1.1	5	5
17	M	79	(15), 12	(L),R	15	0.5	1.0	6	2
18	M	29	3	R	27	0.3	1.5	6	4
19	M	86	35	L+R	30	0.1	1.5	6	2
20	M	75	21	R	31	0	1.4	6	1
21	M	52	2	R	24	0.3	0	5	5
22	F	40	4	R	31	0	0.8	5	2
23	F	33	5	L	36	0.1	0.1	6	6
24	M	37	7	R	31	0.3	0.6	6	2

ND = not determined.

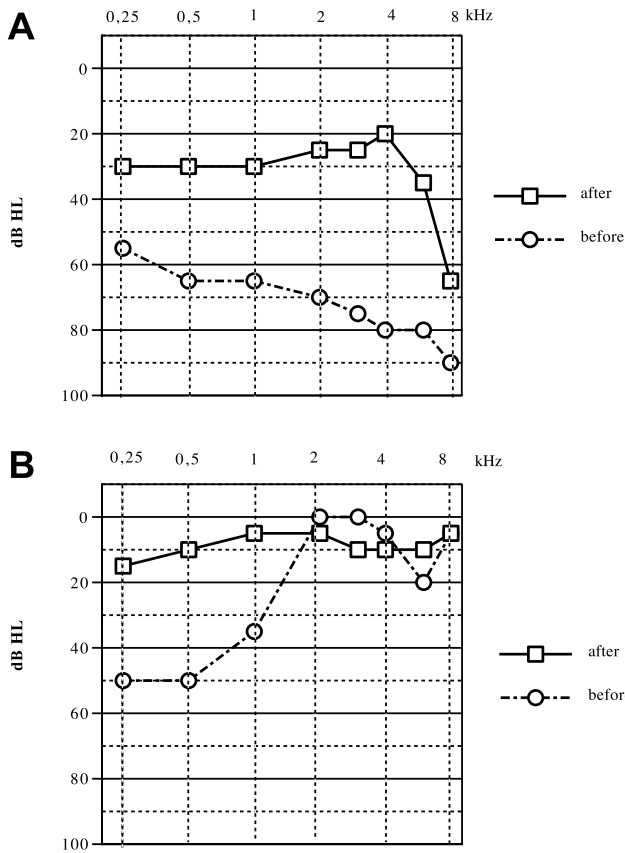


Fig. 1. Audiograms from (A) patient no. 8 and (B) patient no. 9, before and after treatment with SPC.

patients. Three of the patients had bilateral Ménière’s disease, in which case the latest affected ear was studied. Duration of the disease varied from 10 months to 30 years with a mean of 8.4 years.

2.2. Clinical examination

Pure tone and speech audiometry was performed in all patients. In 21 of the 24 patients electronystagmography and oculomotor test had previously been performed as a part of the diagnostic procedures for Ménière’s disease. Pure tone and speech audiometry was done after the diet period.

The extent of vertigo was assessed in each patient before and after the treatment period, using the six-point functional level scale for evaluation of vertigo according to recommendations by AAO-HNS (1995) (Table 1).

2.3. Treatment with SPC

SPC, produced by BioDoc AB (Stockholm, Sweden) and examined as previously described (Björck et al., 2000), were administered to the patients. Each patient received 1 g/kg bw/day of SPC, divided into two to

three dosages over 2–4 weeks. The patients kept a diary during the SPC diet period, noting their subjective symptoms such as fluctuation of hearing and frequency, character and duration of the vertigo. Ten of the 24 patients stopped taking their medication for Ménière’s disease during the trial, while 14 patients continued the medication they normally took.

2.4. Analyses of AF in patients

The plasma samples were drawn before and after the cereal intake period and analyzed as described (Björck et al., 2000). Previous studies on man and animals indicate that AF values of more than 0.5 are correlated with a reduction of diarrheal disease (Lange and Lönnroth, 2001).

2.5. Histology

Two adult male rats, weighing 300 g, were fixed by transcardial perfusion with 4% buffered formaldehyde. The skulls were decalcified in 10% EDTA in Tris-buffered saline and embedded in paraffin. Frontal sections, 4 µm thick, were prepared and sections showing the cochlea and labyrinths were collected. The sections were boiled in a microwave oven and incubated with an affinity-purified antibody raised in rabbits against a 16-amino-acid-long synthetic peptide comprising the active site of AF (Johansson et al., 1997). The antiserum was used at a dilution of 1/5000 to 1/10 000. The immunoreactions were visualized by an alkaline phosphatase-conjugated secondary antibody (Chemicon International, Harrow, UK) and a substrate solution containing nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate toluodim salt (Roche Diagnostics, Mannheim, Germany). Control sections were incubated omitting the primary antibody or with the

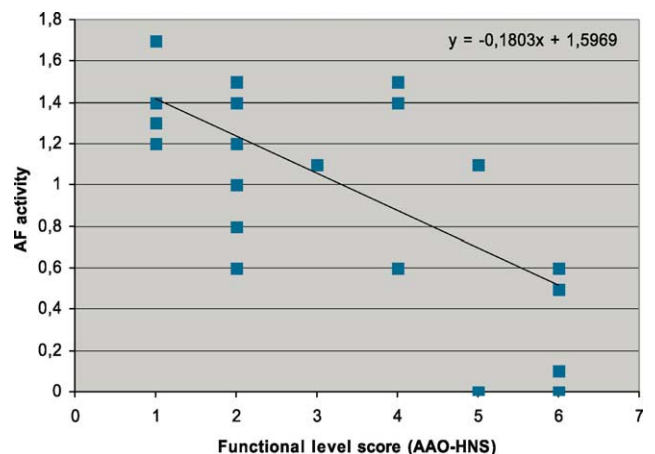


Fig. 2. Correlation between AF activity after treatment and AAO-HNS functional level scale after treatment in the 24 patients. $r = -0.65$; $P < 0.001$.

primary antibody preabsorbed with an excess of the antigen used for immunization. Both types of controls were negative.

2.6. Statistics

Linear regression analyses was performed to explore

the correlation between AF activity and AAO-HNS functional level scale.

2.7. Ethics

The human ethics committee of Göteborg University approved the study design (D. no. H 077-97).

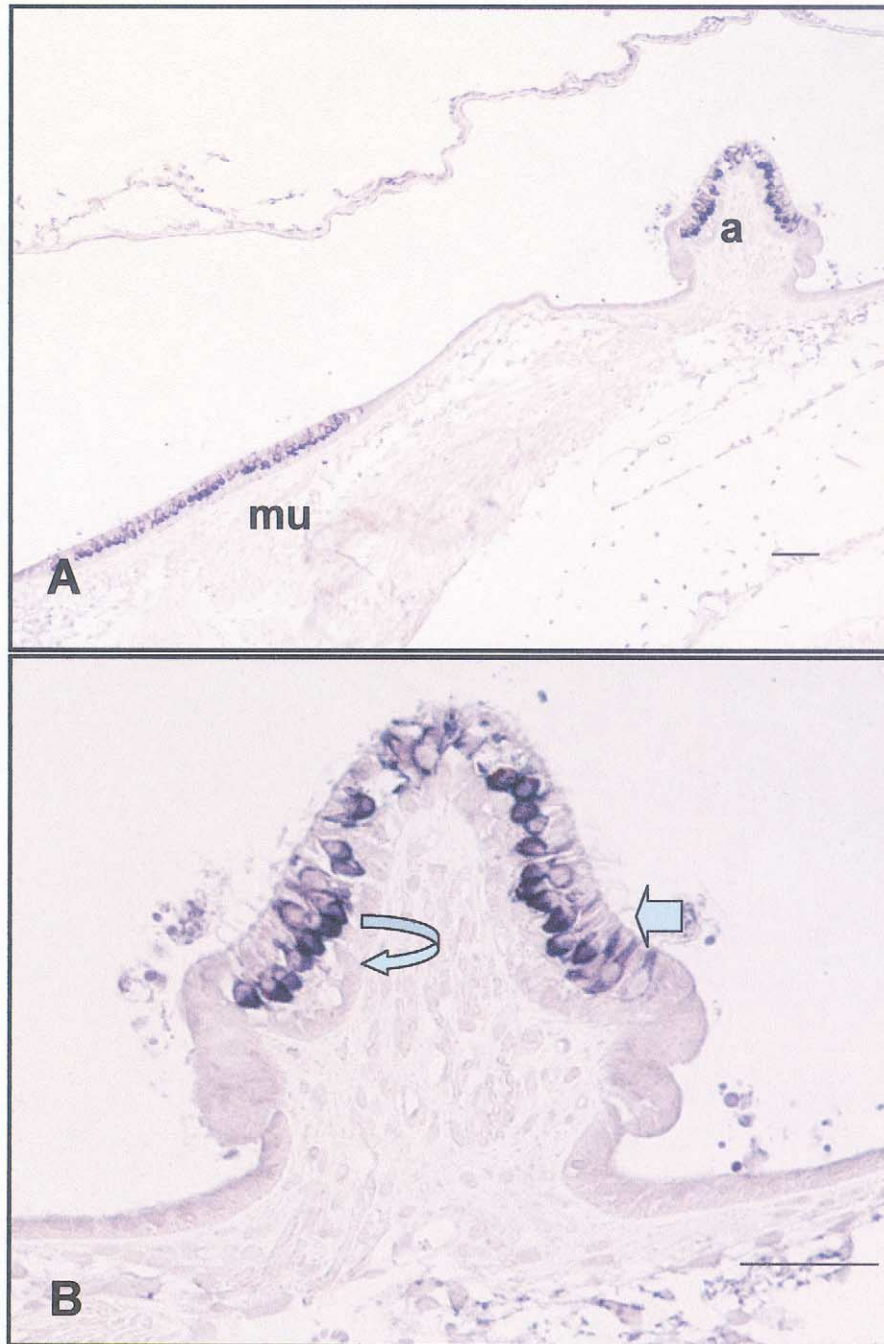


Fig. 3. Section from the inner ear of an adult rat processed to demonstrate AF immunoreactivity. Positive signal appears dark, the sections are not counterstained. Bars = 50 μm. (A) Vestibular apparatus showing crista ampullaris (a) and macula utriculi (mu). (B) Higher magnification of A, showing distinct AF immunoreactivity in the hair cells of crista ampullaris (open arrow). The supporting cells are negative (arrow).

3. Results

3.1. Clinical trial

The results are summarized in [Table 1](#). In 12 patients the attacks of rotatory vertigo were either totally eliminated or changed to more diffuse dizziness (AAO-HNS functional level scale 1–3). These patients had two to five attacks of vertigo weekly over a similar period before the treatment. In three of the patients hearing was normalized and no vertigo attacks occurred following the period of cereal intake. Audiograms from two of these patients are shown in [Figs. 1A,B](#). These patients had three to five attacks of vertigo during a similar period before the treatment with rated severity of 5–6 (mean 5.7). Twelve patients who had two to six attacks weekly before treatment had no or minor effects of the SPC treatment. Their vertigo had rating of 4–6 (mean 5).

3.2. AF analyses

Before SPC treatment, AF plasma activity in blood varied between 0.0 and 0.6 AF units (mean 0.3). After

SPC treatment levels were 0.0–1.7 AF units (mean 0.6) ([Table 1](#)). An increase of more than 0.5 AF units was reached in 20 of the 24 patients.

3.3. Relationship between plasma AF activity and symptoms of Ménière's disease

The correlation between the reduction of vertigo and the AF activity after treatment was statistically significant with a regression coefficient of 0.65 ($P < 0.001$) ([Fig. 2](#)).

Out of the 12 patients who did not respond to the treatment, four patients had no or low AF induction (0.0–0.5, mean 0.15). Eight of the subjects who did not respond had increased AF levels after the treatment (range 0.6–1.6, mean 1.0).

3.4. Histology

In the rat inner ear the most conspicuous AF immunoreactivity was seen in the crista ampullaris and in the macula utriculi ([Fig. 3](#)). In the cochlea AF immunoreactivity was seen in the sensory epithelium of the organ

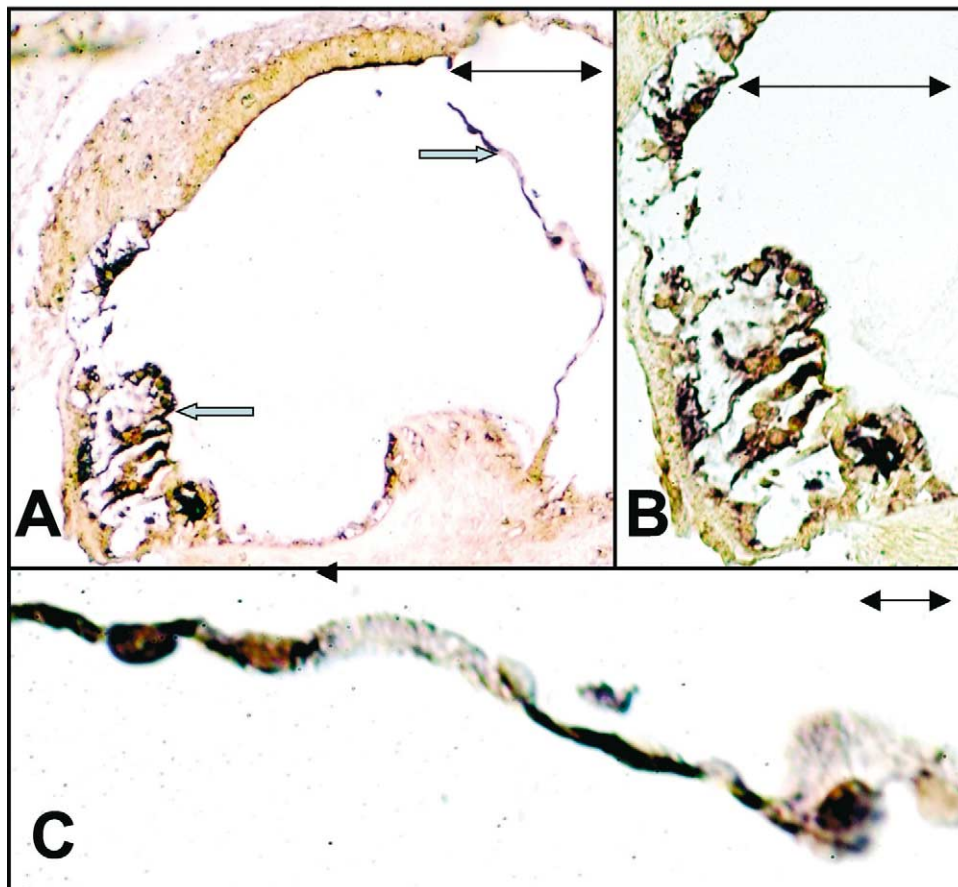


Fig. 4. Section from the cochlea of an adult rat processed to demonstrate AF immunoreactivity. Bars: A and B = 100 μ m, C = 10 μ m. (A) Low magnification showing AF immunoreactivity in the organ of Corti (arrow) and in Reissner's membrane (arrow). (B) Higher magnification showing the organ of Corti. (C). High magnification of Reissner's membrane. AF immunoreactivity is evident in the inner epithelial layer.

of Corti and in Reissner's membrane. Stria vascularis was weakly stained (Fig. 4). Saccus endolymphaticus could not be identified in the investigated sections.

4. Discussion

The pathophysiology of Ménière's disease is poorly understood and consequently it is difficult to predict the mechanisms of treatments that alleviate the symptoms and signs of the disease. There is evidence that the symptoms are related to deficits in the homeostasis of the fluid system of the inner ear. The intracellular localization of AF in cells directly in contact with the endolymph suggests that AF might play a role in the production, modification or regulation of endolymph and that could explain the observed beneficial effect of elevation of AF plasma levels. Disturbances in the normal function or the normal expression of AF could be one aspect of Ménière's disease and explain why AF therapy was successful in 12 out of 24 patients in our study. The fact that immunohistochemistry showed expression of AF in specific cell types facing the endolymphatic spaces in the cochlea and in the vestibular apparatus supports that hypothesis. The present investigation does not, however, explain why the vestibular function generally was better restored than the cochlear in all of the clinically responding patients.

The fact that 12 patients did not experience any clinical improvement from the SPC diet treatment could be caused by insufficient plasma levels of AF. Four of these patients did indeed not have any increase in their plasma AF activity. Absence of clinical improvement could also be the result of lack of compliance, or of an insufficient AF synthesis. Eight patients who had a modest clinical effect of the treatment had a significant AF activity. This may just be another indication of the diversity and complexity of the pathophysiology of Ménière's disease. Previous experiences with other treatment modalities of Ménière's disease (Paparella, 1991; Quaranta et al., 1998) also show that only a certain fraction of patients respond to a specific therapy.

5. Conclusions

The present results show that increasing AF offers an effective treatment for a subpopulation of patients with Ménière's disease, and the study suggests that AF might be involved in homeostasis in the inner ear.

Acknowledgements

Financial support was provided by the Göteborg

Medical Society, AS-Factor AB, Nectin AB, The Swedish State under the LUA agreement (Grant I 33913), the Västra Götaland FoU-fond (KVG-20), Inga-Lisa och Bror Björnsöns Stiftelse, Adlerbertska Forskningsfonden, and the Magnus Bergvall Foundation.

References

- Bagger-Sjöbäck, D., Rask-Andersen, H., 1986. The permeability barrier of the endolymphatic sac. A hypothesis of fluid and electrolyte exchange based on freeze fracturing. *Am. J. Otol.* 7, 134–140.
- Björck, S., Bosaeus, I., Ek, E., Jennische, E., Lönnroth, I., Johansson, E., Lange, S., 2000. Food induced stimulation of the antisecretory factor can improve symptoms in human inflammatory bowel disease: a study of a concept. *Gut* 46, 824–829.
- Committee on Hearing and Equilibrium of the American Academy of Otolaryngology - Head and Neck Foundation, 1995. Guidelines for the diagnosis and evaluation of Ménière's disease. *Otolaryngol. Head. Neck. Surg.* 113, 181–185.
- Göransson, L., Lange, S., Lönnroth, I., 1995. Post weaning diarrhoea: focus on diet. *Pigs News Inf.* 16, 89–91.
- Hallpike, C.S., Cairns, H., 1938. Observations on the pathology of Ménière's syndrome. *J. Laryngol. Otol.* 53, 625–655.
- Ikedo, M., Sando, I., 1984. Endolymphatic duct and sac in patients with Ménière's disease: A temporal bone histopathological study. *Ann. Otol. Rhinol. Laryngol.* 93, 540–546.
- Johansson, E., Lönnroth, I., Lange, S., Jonson, I., Jennische, E., Lönnroth, C., 1995. Molecular cloning and expression of a pituitary gland protein modulating intestinal fluid secretion. *J. Biol. Chem.* 270, 20615–20620.
- Johansson, E., Lange, S., Lönnroth, I., 1997. Identification of an active site in the antisecretory factor protein. *Biochim. Biophys. Acta* 1362, 177–182.
- Kimura, R.S., Ota, C.Y., Schuknecht, H.F., Takahashi, T., 1976. Electron microscopic cochlear observations in bilateral Ménière's disease. *Ann. Otol. Rhinol. Laryngol.* 85, 791–801.
- Lange, S., Lönnroth, I., 2001. The antisecretory factor: synthesis, anatomical and cellular distribution, and biological action in experimental and clinical studies. *Int. Rev. Cytol.* 210, 39–75.
- Nadol, J.B., Jr., Adams, J.C., Kim, J.R., 1995. Degenerative changes in the organ of Corti and lateral cochlear wall in experimental endolymphatic hydrops and human Ménière's disease. *Acta Otolaryngol., Suppl.* 519, 47–59.
- Paparella, M.M., 1991. Methods of diagnosis and treatment of Ménière's disease. *Acta Otolaryngol., Suppl.* 485, 108–119.
- Quaranta, A., Martini, F., Sallustio, V., 1998. Long-term outcome of Ménière's disease: endolymphatic mastoid shunt versus natural history. *Audiol. Neurootol.* 3, 54–60.
- Rask-Andersen, H., Bredberg, G., Lyttkens, L., Loof, G., 1981. The function of the endolymphatic duct—an experimental study using ionic lanthanum as a tracer: a preliminary report. *Ann. NY Acad. Sci.* 374, 11–19.
- Salt, A.N., Thalmann, R., 1988. Interpretation of endolymph flow results: a comment on 'Longitudinal flow of endolymph measured by distribution of tetraethyl ammonium and choline in scala media'. *Hear. Res.* 33, 279–284.
- Salt, A.N., Thalmann, R., Marcus, D.C., Bohne, B.A., 1986. Direct measurement of longitudinal endolymph flow rate in the guinea pig cochlea. *Hear. Res.* 23, 141–151.
- Sterkers, O., Ferrary, E., Amiel, C., 1988. Production of inner ear fluids. *Physiol. Rev.* 68, 1083–1128.
- Yamakawa, K., 1938. Pathologic changes in a Ménière's patient. *Proc. 42nd Meet. Jpn. Otorhinolaryngol. Soc.* 44, 2310–2312.