*The Journal of Laryngology & Otology (2007)*, ***121***, *721–724.*

doi:10.1017/S0022215107005993
Printed in the United Kingdom
First published online 26 February 2007

Main Article

# Distribution and significance of endothelin 1 in guinea pig cochlear lateral wall

D-Y Xu, Y-D Tang, S-X Liu, J Liu*

**Abstract**

Endothelin 1 is a vasoconstrictive peptide with many biological functions. To investigate the distribution of endothelin 1 in guinea pig cochlear lateral wall and the significance of endothelin 1 in maintaining cochlear homeostasis, the immunohistochemistry avidin biotin complex method was applied by using rabbit anti-endothelin 1 polyclonal antibody as primary antibody. Endothelin-1-like activities were detected in the marginal cells, spiral prominence epithelial cells, outer sulcus cells, stria vascularis capillaries, basal cells and spiral ligament fibrocytes.

These results suggest that endothelin 1 may play an important role in maintaining cochlear homeostasis.

**Key words: Endothelin 1; Cochlea; Guinea Pig; Immunohistochemistry**

## Introduction

Endothelin 1 is a vasoconstrictive peptide consisting of 21 amino acids. It was originally isolated from the culture supernatant of porcine aortic endothelial cells by Yanagisawa in 1988.[1] Endothelin 1 is widely distributed in cardiovascular and noncardiovascular tissues. It has many biological functions and plays a significant role in vasoconstriction and the regulation of fluid volume and ion balance. Endothelin-1-like activity has been demonstrated in the inner ear;[2,3] however, the role of endothelin 1 in maintaining cochlear homeostasis is not clear.

The lateral wall of the cochlea is important in the maintenance of cochlear homeostasis. The distribution and significance of endothelin 1 in the cochlear lateral wall requires further study.

In order to understand the microenvironment of the cochlea and how it is maintained, the present study investigated the distribution of endothelin 1 activity in the guinea pig cochlear lateral wall, using immunohistochemistry.

## Materials and methods

Healthy guinea pigs weighing 350–400 g were used. All procedures concerning animals were approved by the animal care and use committee at the Huaxi Medical School of Sichuan University.

After anaesthesia with intraperitoneal pentobarbitone (30 mg/kg) injection, the animals were sacrificed using 200 ml of 0.9 per cent saline administered via cardiac puncture, followed by 4 per cent paraformaldehyde until the neck became stiff.

The temporal bones were removed and fixed overnight (the same fixative was used for all specimens). After decalcification with 10 per cent ethylene diamine triacetic acid for two weeks, the specimens were embedded in paraffin and serially sectioned into 5 μm thick slices. Tissue sections were then deparaffinised, rehydrated, treated with 3 per cent $H_2O_2$ for 10 minutes, immersed in 0.01 M citrate buffer (pH 6.0), microwaved for 5–10 minutes (90–98°C) to retrieve the antigen and then exposed to 5 per cent goat serum for 30 minutes (37°C) to block any nonspecific reaction. Rabbit anti-endothelin 1 polyclonal antibody (diluted 1 : 1200; Peninsula Laboratories, San Carlos, CA, USA) was then added and incubated at 37°C for 120 minutes and at 4°C overnight. Tissue sections were then treated with biotin-labelled goat anti-rabbit immunoglobulin (Ig) G (Beijing Zhongshan, Golden Bridge Biotechnology Co. Ltd., Beijing, China) at 37°C for 60 minutes, followed by adding avidin–biotin–peroxidase complex (Beijing Zhongshan, Golden Bridge Biotechnology Co. Ltd., Beijing, China), incubating at 37°C for 60 minutes, reacting with DAB/$H_2O_2$ solution for 5–10 minutes and then counterstaining with haematoxylin. Specimens were then examined under an Olympus light microscope (Olympus Co. Ltd., Tokyo, Japan).

In the negative control experiment, sections were processed using the same protocol, except that the primary antibody was replaced by phosphate

From the Department of Otorhinolaryngology, and the *Neural Science Center, West China Hospital of Sichuan University, Chengdu, China.
Accepted for publication: 19 April 2006.

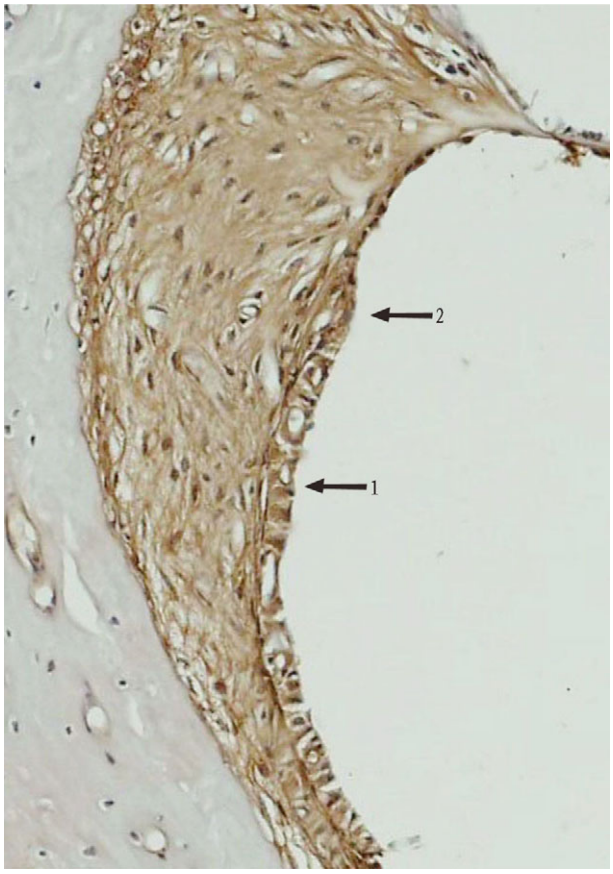


FIG. 1
Endothelin-1-like activity was demonstrated in the stria vascularis (arrow 1), the spiral prominence epithelial cells and outer sulcus cells (arrow 2), and the spiral ligament fibrocytes (ABC; ×200).

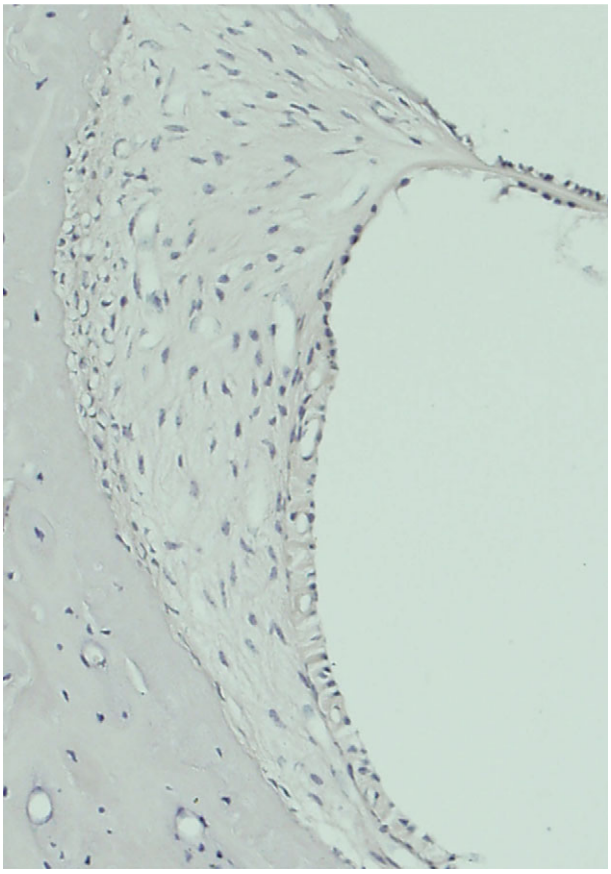

FIG. 2
No immunostaining was observed in the cochlear lateral wall cells from negative control specimens (ABC; ×200).

buffered saline. In the positive control experiment, sections of guinea pig trachea were incubated with the same primary antibody (since endothelin 1 immunoreactivities have been demonstrated in trachea).[4]

## Results

Endothelin-1-like activity was found to be extensively distributed in the cells of the cochlear lateral wall. Different degrees of positive reactivity were demonstrated in the marginal cells, spiral prominence epithelial cells, outer sulcus cells, stria vascularis capillaries, basal cells and spiral ligament fibrocytes (Figure 1). In the negative control, no immunostaining could be found in any area (Figure 2). In the positive control, immunoreactivity was observed in the guinea pig tracheal epithelia, in agreement with published data (Figure 3).

## Discussion

The lateral wall of the cochlea is composed of the stria vascularis, spiral prominence epithelial cells, outer sulcus cells, spiral ligament fibrocytes and capillaries. The cochlear lateral wall plays an important role in production and resorption of endolymph, ion transportation, and generation of the endocochlear potential. Therefore, the cochlear lateral wall is very important in maintaining the homeostasis of the cochlear microenvironment.

The present study showed that endothelin-1-like activity was extensively distributed in the tissues of the cochlear lateral wall, including the marginal cells, basal cells, spiral prominence epithelial cells, outer sulcus cells, spiral ligament fibrocytes and stria vascularis capillaries. These findings indicate

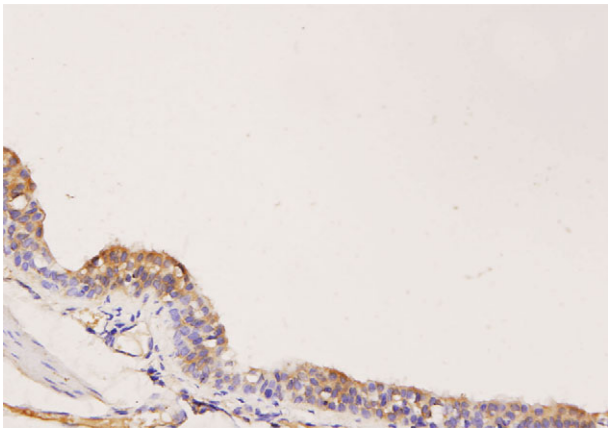

FIG. 3
Immunoreactivity was observed in guinea pig tracheal epithelium (i.e. positive control specimen) (ABC; ×400).

https://doi.org/10.1017/S0022215107005993 Published online by Cambridge University Press

that endothelin 1 may play an important role in the physiological and pathological function of the cochlear lateral wall.

Our findings differ from those reported by Jinnouchi *et al*., who found endothelin-1-like activity to be present only in the marginal cells and not in other cells in the stria vascularis.[2] This may have been due to the different kind of primary antibodies used. The primary antibody used in our study was rabbit anti-endothelin 1 polyclonal antibody, while that used by Jinnouchi *et al*. was mouse anti-human endothelin 1 monoclonal antibody.

The endolymph in the cochlea duct has an ionic composition characterised by large amounts of $K^+$ and small amounts of $Na^+$. A highly positive endocochlear potential has also been found in cochlear endolymph. The unique ionic composition together with the high endocochlear potential are essential to normal hearing. The stability of the ionic composition and the endocochlear potential depend on the active transportation of ions by Na-K-adenosine triphosphatase (ATPase) and Na-K-2Cl, which present in many cells of the cochlear lateral wall.

Marginal cells are located on the surface of the stria vascularis, directly facing the endolymph fluid. The Na-K-ATPase and Na-K-2Cl transporter molecules present in the basolateral plasma membrane of the marginal cells[5,6] work together to transport $K^+$ from the interstitial space and secrete it into the endolymph, across the apical membrane of the marginal cells. The apical membrane of the outer sulcus cells faces the endolymph, and the main functions of these cells include absorbing $K^+$ and $Na^+$ from endolymph and transporting $K^+$ to the spiral ligament via the Na-K-ATPase located in the basolateral plasma membrane.[7,8] The presence of many gap junctions between the basal cells and the type I fibrocytes would be favourable for ion transportation from cell to cell. Some types of fibrocytes in the lateral wall display Na-K-ATPase and Na-K-2Cl transporter molecules,[5,8] providing evidence for a role in ion transportation.

It has been reported that endothelin 1 plays a role in the regulation of Na-K-ATPase and Na-K-2Cl transporter.[9,10] The present study demonstrated endothelin-1-like activity in marginal cells, spiral prominence epithelial cells, outer sulcus cells and spiral ligament fibrocytes. Therefore, we consider that endothelin 1 may regulate ion transportation and influence the generation of endocochlear potential, through its effect on the activity of Na-K-ATPase and Na-K-2Cl transporter.

The stability of endolymph fluid volume is influenced by many factors, including the endolymph flow and ionic composition. Any change in the composition and volume of endolymph will affect normal hearing. The cochlear lateral wall regulates the secretion of $Na^+$ and $H_2O$ and so maintains fluid balance. The present study showed endothelin-1-like activity to be extensively distributed amongst the cells of the lateral wall. This suggests that endothelin 1 might affect the homeostasis of the cochlear endolymph volume.

- **Endothelin 1 is a vasoactive peptide with many biological functions**
- **This study investigated the distribution of endothelin 1 in the guinea pig cochlea**
- **Endothelin 1 is widely distributed in the guinea pig cochlea, suggesting that it may have a role in maintaining cochlear haemostasis**

Atrial natriuretic peptide is a diuretic, natriuretic hormone. This hormone has been reported to be extensively distributed in the stria vascularis.[11] The present study shows endothelin 1 to be localized in the same regions as atrial natriuretic peptide. This supplies evidence that endothelin 1 stimulates the release of atrial natriuretic peptide.[12] We therefore suggest that the endothelin 1 of these cells may affect the homeostasis of endolymph through its action on atrial natriuretic peptide.

## Conclusion

Endothelin 1 is extensively distributed in the cells of the cochlear lateral wall and may function as an important regulator for maintaining the homeostasis of the cochlear microenvironment. However, the mechanism of action of endothelin 1 requires further study.

## Acknowledgements

We should like to thank Dr Hui-Min An for counsel and advice. This project was supported by the Natural Science Foundation of China (30271408).

### References

1 Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y *et al*. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988;**332**:411–15
2 Jinnouchi K, Tomiyama S, Pawankar S, Ikezono T, Yagi T. Distribution of endothelin-1-like activity in the cochlea of normal guinea pigs. *Acta Otolaryngol* 1997; **117**:41–5
3 Jinnouchi K, Tomiyama SI, Pawankar R. Distribution of endothelin-1-like activity in the vestibule of normal guinea pigs. *ORL J Otorhinolaryngol Relat Spec* 1996;**58**: 4–8
4 Kizawa Y, Kotake H, Kusama T, Saito K, Murakami H. Antigen-induced elevation of immunoreactive endothelin-1 (ET-1) levels in ovalbumin-sensitized guinea pig airway tissue. *Comp Biochem Physiol C Pharmacol Toxicol Endocrinol* 1999;**122**:239–43
5 Goto S, Oshima T, Ikeda K, Ueda N, Takasaka T. Expression and localization of the Na-K-2Cl cotransporter in the rat cochlea. *Brain Res* 1997;**765**:324–6
6 Nakazawa K, Spicer SS, Schulte BA. Ultrastructural localization of Na,K-ATPase in the gerbil cochlea. *J Histochem Cytochem* 1995;**43**:981–91
7 Marcus DC, Chiba T. $K^+$ and $Na^+$ absorption by outer sulcus epithelial cells. *Hear Res* 1999;**134**:48–56
8 Schulte BA, Adams JC. Distribution of immunoreactive $Na^+$-$K^+$-ATPase in gerbil cochlea. *J Histochem Cytochem* 1989;**37**:127–34
9 Prasanna G, Dibas A, Hulet C, Yorio T. Inhibition of Na(+)/K(+)-ATPase by endothelin-1 in human

https://doi.org/10.1017/S0022215107005993 Published online by Cambridge University Press

nonpigmented ciliary epithelial cells. *J Pharmacol Exp Ther* 2001;**296**:966–71
10 Gupta S, Ruderman NB, Cragoe EJ Jr, Sussman I. Endothelin stimulates Na(+)-K(+)-ATPase activity by a protein kinase C-dependent pathway in rabbit aorta. *Am J Physiol* 1991;**261**(1Pt2):H38–45
11 Chen HX, Wang JL, Liu QC, Qiu JH. Distribution and location of immunoreactive atrial natriuretic peptides in cochlear stria vascularis of guinea pig. *Chin Med J (Engl)* 1994;**107**:53–6
12 Skvorak JP, Nazian SJ, Dietz JR. Endothelin acts as a paracrine regulator of stretch-induced atrial natriuretic peptide release. *Am J Physiol* 1995;**269**(5Pt2):R1093–8

Address for correspondence:
Yue-Di Tang,
Department of Otorhinolaryngology,
West China Hospital of Sichuan University,
Chengdu, China 610041.

E-mail: tangyd@hotmail.com

Dr Y-D Tang takes responsibility for the integrity of the content of the paper.
Competing interests: None declared