Oxygen modulates Na\(^+\) absorption in middle ear epithelium


Oxygen modulates Na\(^+\) absorption in middle ear epithelium. Am. J. Physiol. 276 (Cell Physiol. 45): C312–C317, 1999.—The physiology of the middle ear is primarily concerned with keeping the cavities air filled and fluid free to allow transmission of the sound vibrations from the eardrum to the inner ear. Middle ear epithelial cells are thought to play a key role in this process, since they actively transport Na\(^+\) and water. The PO\(_2\) of the middle ear cavities varies from 44 to 54 mmHg in healthy human ears but may be lower in the course of secretory otitis media. The effect of chronic hypoxia on ion transport was investigated on a middle ear cell line using the secretory otitis media assay. The decrease of Na\(^+\) absorption across the MESV cell line. Although a decrease in cellular ATP content was observed, the decrease of Na\(^+\) absorption seemed related to a primary modulation of apical Na\(^+\) entry. As revealed by RNase protection assay, the decrease in apical Na\(^+\) entry strictly paralleled the decrease in the expression of transcripts encoding the \(\alpha\)-subunit of the epithelial Na\(^+\) channel. This effect of oxygen on Na\(^+\) absorption might account for 1) the presence of fluid in the middle ear in the course of secretory otitis media and 2) the beneficial effect of the ventilation tube in treating otitis media that allows the PO\(_2\) to rise and restores the fluid clearance.

THE PHYSIOLOGY OF THE middle ear is primarily concerned with keeping the cavities air filled and fluid free, to allow transmission of the sound vibrations from the eardrum to the inner ear. Middle ear epithelial cells are thought to play a key role in this process. They eliminate mucus from the tympanic cavity by means of apical cilia (1) and also actively transport Na\(^+\) and water to clear any fluid present in excess (9, 20).

The perfusion of the mucosa is thought to be poor, since high Pco\(_2\) and low PO\(_2\) have been reported in the middle ear cavities (18). Therefore, as far as oxygen is concerned, epithelial cells may rely not only on the capillary bed but also on the gas mixture of the middle ear cavities. This gas mixture has long been thought to originate from boluses of exhaled air, since the middle ear communicates with the pharynx through the eustachian tube. This would likely allow the PO\(_2\) to reach 120 mmHg (17). However, several recent works have reported that the middle ear gas composition differs dramatically from that of atmospheric air and is similar to the composition of mixed venous blood (11, 26, 35). This might be related to the very short openings of the eustachian tube. The PO\(_2\) varies from 44 mmHg (18) to 54 mmHg (15) in healthy human ears but may be lower in the course of secretory otitis media, from 31 to 51 mmHg (24). The middle ear cells are thus putatively exposed to a minor hypoxia.

Hypoxia is central to many pathophysiological disorders. Most mammalian cells are very sensitive to the decrease in PO\(_2\), which may lead to irreversible cellular damage (13). However, a certain respiratory epithelium was shown to be quite resistant to prolonged hypoxia, with a reversible modulation of the rate of ion transport (33). Because the middle ear epithelium is also of respiratory type, the evaluation of middle ear epithelial cell function under hypoxia represents an important issue.

In this work, we report that chronic hypoxia reversibly decreased the rate of Na\(^+\) absorption across middle ear epithelial cells. Although a decrease in cellular ATP content was observed, the decrease of Na\(^+\) absorption seemed related to a primary modulation of apical Na\(^+\) entry. A decrease in the expression of transcripts encoding the \(\alpha\)-subunit of the epithelial Na\(^+\) channel (\(\alpha\)-ENaC) was evidenced, which paralleled the decrease of Na\(^+\) absorption.

MATERIALS AND METHODS

Cell culture. Techniques have been described elsewhere (23). The MESV cell line was previously established from Mongolian gerbil (Meriones unguiculatus) middle ear epithelial primary culture by infection with simian virus 40 (20). Briefly, MESV cells were regularly subcultured in a humidified 5% CO\(_2\) incubator at 37°C, in a medium composed of DMEM-medium 199 (1:1 vol/vol) containing 5% FCS, 10 ng/ml epidermal growth factor, 5 µg/ml transferrin, 2 nM triiodothyronine, 5 µg/ml insulin, 10^{-6} M hydrocortisone, 10^{-7} M dexamethasone, 100 U/ml penicillin, 100 µg/ml streptomycin, 15 mM HEPES, and 2 mM L-glutamine.

For electrophysiological measurements, confluent MESV monolayers were trypsinized and plated (10^3 cells/cm\(^2\)) onto tissue culture-treated polycarbonate filters (0.4-µm pore, 1 cm\(^2\); Snapwell, Costar, Cambridge, MA).

Hypoxic exposure. To achieve hypoxic exposure, culture dishes were placed in a humidified airtight incubator with inflow and outflow valves, and the desired hypoxic gas mixture (0% O\(_2\)-5% CO\(_2\)-95% N\(_2\), 5% O\(_2\)-5% CO\(_2\)-90% N\(_2\), or 10% O\(_2\)-5% CO\(_2\)-85% N\(_2\)) was delivered at a constant flow rate for 20 min. The airtight incubator was kept at 37°C for 1, 3, 6, 12, 18, or 24 h, while normoxic control cells were placed in a 21% O\(_2\)-5% CO\(_2\)-74% N\(_2\) humidified incubator for the same period of time. In our conditions, values of PO\(_2\) assayed in the...
culture medium (mPO2) were 32, 50, 100, and 150 mmHg after 18-h incubation with 0, 5, and 10% O2 (hypoxia) and 21% O2 (normoxia), respectively.

Morphology. To investigate the morphological impact of hypoxia exposure on MESV cells, cells were incubated 18 h in normoxia or hypoxia. Afterwards, cells were rinsed with cacodylate buffer (0.1 M) and fixed overnight with 2.5% glutaraldehyde. Specimens were then washed in buffer, post-fixed with 1% OsO4, dehydrated, and embedded in Epon. The sections were counterstained with uranyl acetate and further processed for transmission electron microscopy (EM 410, Philips, Eindhoven, The Netherlands).

Determination of cellular protein content. The method for the quantification of the protein content in MESV cells utilized the principle of protein-dye binding (5). BSA was used as standard. Results are expressed in micrograms of protein per well or per filter.

Bioelectric measurements. Cells were used 5 days after seeding. Filters were mounted into micro-Ussing chambers perfused with medium. The perfusion medium was lifted with seeding. Filters were mounted into micro-Ussing chambers (UK). Short-circuit conditions were maintained throughout experimentation in MgSO4, 0.15 Na2HPO4, 0.2 NaH2PO4, 4 NaHCO3, 1 CaCl2,5 mmol/L d-glucose. Further incubation conditions were maintained throughout the experiment, and the short-circuit current (Isc) was continuously recorded on a pen chart recorder (Servotrace, Sefram, Paris, France). Every 30 s, voltage was clamped at 1 mV, so that the transepithelial resistance (Rt) could be determined by Ohm's law. Two currents were studied: 1) Isc across MESV monolayers and 2) IscNa, the fraction of Isc sensitive to benzamil (10−5 M), a highly selective apical Na+ channel inhibitor (36).

Determination of intracellular ATP content. Intracellular ATP content was determined with luciferase assay according to Doctor et al. (10). Cells were cultured on six-well plates for 4 days and then incubated for 18 h in normoxia or 1, 3, 6, or 18 h in hypoxia. The cells were washed, and ice-cold 3% (3 N) perchloric acid was added. After 3 min, the monolayer was scraped from the dish and the resulting mixture was centrifuged (10 min at 2,000 rpm). The pellets were resuspended in 0.1 N NaOH for protein determination. The supernatant was neutralized with KOH and stored at −80°C until protein determination. ATP content of the supernatant was measured in a luminometer (Hewlett-Packard Picolite lumino-meter, Packard Instrument) using an ATP determination kit (Calbiochem). Standard curves of log photons vs. log ATP were linear over the range from 10−8 to 10−4 M ATP. Results were expressed in micromoles of ATP per milligram of protein. Each data point is the mean of three measurements.

Measurement of the ouabain-sensitive 86Rb+ uptake. The ouabain-sensitive Rb+ influx, determined by the difference between 86Rb+ uptake with and without ouabain, was used as an indicator of Na+−K+−ATPase activity (8). MESV cells were incubated for 18 h in normoxia or hypoxia, with or without benzamil in the apical bath (10−5 M). Uptake measurements were performed at 37°C in a solution derived from Eagle's essential medium containing (in mM) 120 NaCl, 5 RbCl, 1 MgSO4, 0.15 Na2HPO4, 0.2 NaH2PO4, 4 NaHCO3, 1 CaCl2, 5 glucose, and 20 HEPES (pH 7.4). The osmolality was adjusted with mannitol to 350 mosmol/kg. Cells were preincubated in the presence of ouabain (5 mM) or vehicle, and then uptake was performed for 5 min with 86Rb+ (2 µCi/ml) in the basolateral compartment. Uptake solutions used on hypoxic cells were preoxygenated with the hypoxic gas to avoid reoxygenation during the uptake procedure. Uptake was stopped by washing the cells three times with ice-cold buffer. Radioactivity was then extracted by Triton X-100 (1%) and counted in a scintillation counter. The protein content of each filter was determined, and results were expressed as picograms of 86Rb per filter.

RESULTS

Morphology. Dome formation is a constant feature of MESV cells when grown on nonporous supports. Surprisingly, incubation for 18 h in hypoxic conditions dramatically prevented dome formation.

Transmission electron microscopy did not reveal any morphological differences, such as cellular hypertrophy, increase in cell height, or amplification of basolateral areas, between cells grown in normoxia and 18-h hypoxia. Neither cilia nor secretory granules could be observed in either condition.

Effect of hypoxia on Isc. Incubation of cell monolayers in a hypoxic medium (mPO2 32 mmHg) induced a time-dependent decrease of Isc from 3.21 ± 0.57 µA/cm2...
in normoxia (control; n = 12) to 1.28 ± 0.22 µA/cm² (P < 0.01; n = 8) after 18-h hypoxia. The Rₑ was not modified by hypoxia [1.623 ± 0.154 Ω·cm² for control vs. 1.334 ± 0.141 Ω·cm² after 18-h hypoxia; not significant (NS); n = 10].

The Iₛₐ decreased paralleled that of Iₛ,Na; 1) Iₛ,Na decreased in a time-dependent manner from 1.71 ± 0.23 µA/cm² in control (n = 12) to 0.64 ± 0.10 µA/cm² (P < 0.01; n = 8) after 18-h hypoxia, and 2) the Iₛ and Iₛ,Na fit curves (1-phase exponential decay, R² = 0.91 for Iₛ and R² = 0.98 for Iₛ,Na) were parallel (Fig. 1).

The dose-effect relationship of this process was evaluated. Cells were incubated in various hypoxic conditions. Iₛ,Na decreased significantly with mPO₂: 1.39 ± 0.14 µA/cm² (n = 4) for control (mPO₂ 150 mmHg) and 1.45 ± 0.06 (NS; n = 3), 1.10 ± 0.07 (P < 0.05; n = 3), and 0.73 ± 0.07 µA/cm² (P < 0.01; n = 3) for mPO₂ of 100, 50, and 32 mmHg, respectively (Fig. 2).

The hypoxia-induced decrease of Iₛ,Na in MESV cells was partly reversible. In 6- or 18-h hypoxic cells (mPO₂ 32 mmHg), Iₛ,Na was not significantly different from control after a 6-h reoxygenation (Fig. 3): 1.85 ± 0.6 M benzamil (10⁻⁶ M benzamil) was subsequently measured. Values are means ± SE of Iₛ,Na (n = 3–4). *Significantly different from control (P < 0.05; n = 3–4).

Measurement of ouabain-sensitive ⁸⁶Rb⁺ uptake. Incubation for 18 h in hypoxia dramatically decreased the ouabain-sensitive ⁸⁶Rb⁺ uptake from 164.99 ± 2.63 pg ⁸⁶Rb⁺/filter for normoxic cells to 38.61 ± 1.06 pg ⁸⁶Rb⁺/filter for hypoxic cells (P < 0.001; n = 3). To evaluate the target of hypoxia in our system, either apical or basal, we measured ouabain-sensitive Rb⁺ influx with or without benzamil (10⁻⁶ M) in the apical bath. Our results show that benzamil decreased ouabain-sensitive Rb⁺ influx in normoxic cells (91.71 ± 8.67 pg ⁸⁶Rb⁺/filter; P < 0.01; n = 3). However, hypoxic and benzamil effects on ouabain-sensitive Rb⁺ influx were not additive, as shown in Fig. 4. In hypoxic cells, benzamil did not further decrease ouabain-sensitive Rb⁺ influx (47.73 ± 10.16 pg ⁸⁶Rb⁺/filter with benzamil vs. 38.61 ± 1.06 pg ⁸⁶Rb⁺/filter without benzamil; NS; n = 3).

**Fig. 1.** Time-response relationship for effect of hypoxia on short-circuit current (Iₛ) of MESV monolayers. Iₛ and fraction of Iₛ sensitive to apical 10⁻⁶ M benzamil (Iₛ,Na) were measured for normoxic cells (0 h; 150 mmHg PO₂) and after 1, 3, 6, 12, and 18-h hypoxia (32 mmHg mPO₂). Recorded values were fitted by a 1-phase exponential decay: R² = 0.91 for Iₛ; R² = 0.98 for Iₛ,Na (Graph Pad Prism, Graph Pad Software, San Diego, CA). Values are means ± SE. *Significantly different from control (P < 0.05; n = 8–12).

**Fig. 2.** Dose-response relationship for effect of hypoxia on Iₛ,Na of MESV monolayers. MESV monolayers were incubated for 18 h in normoxic conditions (150 mmHg mPO₂) or hypoxic conditions (100, 50, or 32 mmHg mPO₂), and benzamil-sensitive Iₛ,Na (10⁻⁶ M benzamil) was subsequently measured. Values are means ± SE of Iₛ,Na (n = 3–4). *Significantly different from control (P < 0.05; n = 3–4).

**Fig. 3.** Reversibility of hypoxia effect on Iₛ,Na of MESV monolayers. Iₛ,Na was measured in normoxic cells (150 mmHg mPO₂; open bars), in 6- or 18-h hypoxic cells (32 mmHg mPO₂; hatched bars), and in 6-h reoxygenated cells (solid bars). Values are means ± SE of Iₛ,Na (n = 3–4). *Significantly different from control (P < 0.05; n = 3–4); §Significantly different from control (P < 0.05; n = 3).

**Fig. 4.** Effect of ouabain-sensitive ⁸⁶Rb⁺ influx with or without benzamil (10⁻⁶ M) in the apical bath. Our results show that benzamil decreased ouabain-sensitive Rb⁺ influx in normoxic cells (91.71 ± 8.67 pg ⁸⁶Rb⁺/filter; P < 0.01; n = 3). However, hypoxic and benzamil effects on ouabain-sensitive Rb⁺ influx were not additive, as shown in Fig. 4. In hypoxic cells, benzamil did not further decrease ouabain-sensitive Rb⁺ influx (47.73 ± 10.16 pg ⁸⁶Rb⁺/filter with benzamil vs. 38.61 ± 1.06 pg ⁸⁶Rb⁺/filter without benzamil; NS; n = 3).
Table 1. Intracellular ATP content

<table>
<thead>
<tr>
<th>Cell Culture Condition</th>
<th>ATP Concentration, µM/ATP/mg protein</th>
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<tbody>
<tr>
<td>Normoxia</td>
<td>126.6 ± 5.8</td>
</tr>
<tr>
<td>Hypoxia 1 h</td>
<td>138.2 ± 4.9</td>
</tr>
<tr>
<td>Hypoxia 3 h</td>
<td>103.2 ± 4.8*</td>
</tr>
<tr>
<td>Hypoxia 6 h</td>
<td>37.8 ± 1.9*</td>
</tr>
<tr>
<td>Hypoxia 18 h</td>
<td>64.2 ± 3.2*</td>
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Values are means ± SE of ATP values; n = 12 measurements. Intracellular ATP content was determined by luciferin-luciferase assay in normoxic and 1-, 3-, 6-, or 18-h hypoxic monolayers. *Statistically different from normoxia (P < 0.01).

Effect of hypoxia on α-ENaC subunit mRNA levels. RNase protection assays were performed to evaluate the expression of α-ENaC subunit mRNA transcripts in normoxic and hypoxic MESV cells. β-Actin expression was used as an internal standard, since the level of β-actin mRNA was not significantly modified by hypoxia (11.73 ± 0.96 cpm for control cells vs. 9.95 ± 1.77 cpm for hypoxic cells; NS; n = 7–8). Exposure of MESV cells to hypoxia for 18 h induced a 50% decrease in the expression of α-ENaC mRNA (67.5 ± 7.7 vs. 135.3 ± 13.25 AU for normoxic cells; P < 0.05; n = 5–8). This decrease paralleled the decrease of Isc,Na (63%) observed in the Isc assays. When hypoxic cells were allowed to recover in 150 mmHg O2, the level of α-ENaC mRNA transcripts was not different from control (116.3 ± 12.46 AU; NS; n = 5–8; Fig. 5).

DISCUSSION

This work reports a reversible hypoxia-induced decrease of Na+ absorption in middle ear epithelial cells. This modulation was likely related to a reversible decrease in the expression of the apical Na+ channel mRNA rather than to metabolic effects of hypoxia.

Hypoxia decreases the rate of Na+ absorption in the middle ear epithelium. Ion transport activity of the middle ear epithelium has been previously studied in primary cultured cells (12, 19) and in the MESV cell line (20) by the Isc technique. In these cells, most of the electrogenic ion transport is related to an active absorption of Na+ from the luminal to the basolateral compartment, occurring through apical amiloride-sensitive Na+ channels. This process is thought to drive a water flow and to contribute to the maintenance of air-filled and fluid-filled cavities. The Na+ absorption was herein evaluated as Isc,Na (22). Chronic hypoxia induced a time-dependent decrease of Isc in MESV cells (~60% after 18 h of exposure). This hypoxia-induced decrease paralleled the decrease in Na+ absorption, as evaluated by Isc,Na (~63% after 18 h of exposure; see Fig. 1). The fraction of Isc insensitive to benzamil may be related to an apical Cl– channel (12, 21). Because of an unfavorable electrochemical gradient, this Cl– channel seems to be active only when Na+ entry is blocked (12), as in the case of human nasal epithelial cells (4).

In numerous cell types, decrease of PO2 induces irreversible damage (6, 13). However, hypoxia was also shown to induce specific nontoxic effects, such as modulation of ion transport in lung epithelial cells (31, 32) or cardiomyocytes (14). In MESV cells, the decrease in Na+ absorption was not related to irreversible cellular damage: 1) ultrastructural study did not reveal cellular modifications or damage; 2) continuous measurement of Rr, attested for the absence of leak in the monolayers, and 3) the hypoxia-induced effects on Isc,Na were reversible after reoxygenation.

Hypoxia promotes a decrease of the expression of transcripts encoding α-ENaC. The PO2 variations have been shown to affect ion transport function. Depending on the model, hypoxia modulates apical ion channels (31, 32) and/or basolateral Na+–K+–ATPase activity (16, 29, 32). To characterize the target of hypoxia in our system, the Na+–K+–ATPase activity was measured by the ouabain-sensitive 86Rb+ uptake, with or without a benzamil-induced apical Na+ entry inhibition. We observed that hypoxia induced a 75% decrease in Rb+ uptake.

Fig. 4. Effect of hypoxia on ouabain-sensitive 86Rb+ uptake (OsRb). Na+–K+–ATPase activity was determined as the 5 mM ouabain-sensitive 86Rb+ uptake in normoxic or 18-h hypoxic monolayers (32 mmHg mPO2) with or without benzamil (Bz; 10–4 M) in apical bath during incubation period. *Significantly different from control (P < 0.05; n = 3).

Fig. 5. Modulation by hypoxia of expression of epithelial Na+ channel α-subunit (α-ENaC) mRNA transcripts. RNase protection assay was performed on cell lysis from normoxic (150 mmHg mPO2; CTL) and hypoxic (32 mmHg mPO2; Hypoxia) MESV monolayers. Latter were incubated for 18 h under hypoxic conditions, eventually followed by a 6-h reoxygenation (Reox). Antisense RNA probe was synthesized from translated region of human α-ENaC. β-Actin was used for standardization. *Significantly different from control (P < 0.05; n = 5–8). §Significantly different (P < 0.05; n = 8).
uptake, i.e., in Na\(^+\)-K\(^+\)-ATPase activity. This could have been related to an effect of hypoxia either on apical Na\(^+\) entry or on basal extrusion through the pump. If the only target of hypoxia were the basal pump, one would expect the addition of benzamil, which blocks the apical Na\(^+\) entry, to reduce the intracellular Na\(^+\) concentration and, as a result, the activity of the basal pump. In that case, hypoxia combined with benzamil should yield a lower Na\(^+\)-K\(^+\)-ATPase activity than hypoxia alone, which was not the case. This result suggests that the actual target of hypoxia was the apical Na\(^+\) entry, which does not preclude a simultaneous effect on the basal pump. Furthermore, hypoxia significantly decreased \(I_{sc,Na}\) after 12 h, a delay compatible with a transcriptional effect. For these reasons, the hypoxia-induced modulation of the expression of transcripts encoding ENaC was measured. The ribonuclease protection assay was performed with a probe synthesized from the translated region of the \(\alpha\)-subunit (bp 1036–1259) of the hENaC (\(\alpha\)-ENaC). Although some interspecies differences have restrained the size of the protected fragment, examination of rat and human sequences reveals that the translated region used is highly maintained, with a 92% homology, the longest identical sequence being 144 bp long (22). Hypoxia decreased by 50% the expression of \(\alpha\)-ENaC subunit transcripts. Although posttranscriptional events might affect the effective production of Na\(^+\) channels, this result paralleled the hypoxia-induced decrease of the transepithelial Na\(^+\) absorption observed in functional assays (\(I_{sc,Na}\) decreased by 60%). The decrease of \(\alpha\)-ENaC mRNA transcripts was not related to irreversible cellular damage, since 1) \(\beta\)-actin mRNA transcripts were not modified by hypoxia and 2) after an 18-h hypoxia, reoxygenation allowed parallel \(I_{sc,Na}\) and \(\alpha\)-ENaC mRNA recovery. Hypoxia-induced decrease in Na\(^+\) absorption might also have been related to downregulation of \(\beta\) and \(\gamma\)-subunits. However, it should be stated that the \(\alpha\)-subunit of the Na\(^+\) channel exhibits, when expressed in oocytes, all the characteristics of the highly selective channel, whereas \(\beta\) and \(\gamma\)-subunits only allow maximal activity of active Na\(^+\) channels (7, 36).

Hypoxia-induced Na\(^+\) absorption modulation may not only occur through \(\alpha\)-ENaC mRNA transcript modulation. It has been shown in other tight epithelia that the rate-limiting step for transepithelial Na\(^+\) reabsorption is Na\(^+\) entry (34), whereas Na\(^+\)-K\(^+\)-ATPase adapts its activity to maintain a low intracellular Na\(^+\) concentration (3). The data we present strongly support a downregulation of \(\alpha\)-ENaC mRNA. However, translation or posttranslational hypoxia-induced regulation cannot be excluded because 1) neither the Na\(^+\) channels produced nor those actually present on the apical cellular membrane were quantified, and 2) hypoxia might modulate ion transport function because of cytosolic factors such as intracellular Ca\(^2+\) increase (2, 32) or intracellular ATP decrease (28). Actually, a significant decrease in cellular ATP content was observed in hypoxic conditions in MESV cells. A 70% decrease after a 6-h incubation was followed by a partial recovery for a longer incubation time (49% at 18 h), which may be related to an activation of anaerobic metabolism. This decrease may contribute to the effect of hypoxia on Na\(^+\) absorption. However, the Michaelis constant of the Na\(^+\)-K\(^+\)-ATPase for ATP is very low, so that only drastic depletion can affect the Na\(^+\)-K\(^+\)-ATPase activity (25). Last, although the Na\(^+\)-K\(^+\)-ATPase activity decrease seemed to be related to a decrease of apical Na\(^+\) entry and apico-basal coupling, a transcriptional modulation cannot be excluded.

Pathophysiological incidence of the epithelial effect of hypoxia-reoxygenation. Some in vivo experiments (31, 36) have indicated that \(P_{CO_2}\) may be high in the middle ear cavities (~60 mmHg). Because of the high diffusion rate coefficient of CO\(_2\), this fact suggests that the perfusion of the mucosa is poor and that cellular metabolism may rely on the O\(_2\) content of the middle ear cavities. This oxygen content, measured as the middle ear P\(_{O_2}\), is physiologically near 50 mmHg (15, 18). In pathological conditions, the middle ear P\(_{O_2}\) may further decrease because of eustachian tube dysfunction or vascular modifications, although available data are scarce and controversial (19, 24).

Our data demonstrate that hypoxia dramatically downregulates the epithelial Na\(^+\) absorption and thus the osmotically induced water flux from the apical to the basolateral side in the middle ear epithelium. As far as these in vitro experimental data can be extrapolated to the in vivo situation, the oxygen-induced modulation of Na\(^+\) absorption might account for 1) fluid excess in the course of secretory otitis media and 2) the beneficial effect of the ventilation tube in treating otitis media because of a reoxygenation-induced improvement in fluid clearance from the middle ear cavities.

We are grateful to Richard Boucher for kindly providing the \(\alpha\)-hENaC subunit cDNA. We are indebted to Christine Clerici and Carole Planès for helpful discussion.

This work was supported by grants from Fondation pour la Recherche Médicale (France).

Address for reprint requests: F. Portier, Hopital Lariboisière, Service ORL 2, Rue Ambroise Pare´, 75010 Paris, France.

Received 4 March 1998; accepted in final form 21 October 1998.

REFERENCES

C317