Send Orders for Reprints to reprints@benthamscience.ae

Open Access

Regulation of Cochlear Fluids pH: (I) The Role of Carbon Dioxide

Masaaki Suzuki*, Tadashi Wakayama and Ryosuke Kotani

Department of Otolaryngology, Teikyo University Chiba Medical Center, Chiba, Japan

Abstract: Although bicarbonate and hydrogen ions (pH) are known to play significant roles in the physiology of the cochlea, the details of these interrelationships have only received limited attention. Since bicarbonate ions represent the predominant pH buffer, one factor which profoundly affects pH in the cochlea is the prevailing level of carbon dioxide. In the present study, endocochlear potential (EP), endolymph pH (PH_e) and scala vestibuli perilymph pH (PH_v) were monitored in the second tum of the guinea pig cochlea during manipulations of the systemic CO_2 level. Hypercapnea and hypocapnea were induced by varying the CO_2 content of inspired air while the animal was slightly hyperventilated. Hypercapnea decreased PH_e and PH_v and increased the EP, while hypocapnea produced opposite results. In separate studies, endolymph potassium concentration (K^+_e) was found to be decreased by hypercapnea and increased by hypocapnea. No systematic changes of endolymph Cl^-or Na^+ were found. CO_2-induced EP, pH and K_e changes were virtually abolished by intravenous administration of the carbonic anhydrase inhibitor, acetazolamide. These findings support the view that HCO_3^-/CO_2 buffering of pH plays a prominent role in the pH regulation of cochlear fluids and may play a key role in endolymph electrolyte homeostasis.

Keywords: Cochlear fluids pH, endocochlear potential, endolymph potassium concentration

INTRODUCTION

Many cellular processes exhibit significant sensitivity to the pH of the bathing medium. In an *in vitro* preparation of isolated vestibular dark cells it was demonstrated that the voltage and current across the tissue increased as extracellular pH decreased, and decreased as extracellular pH increased [1]. The magnitude of the extracellular pH changes required to affect ion transport profoundly was small, with an increase in pH to about 7.6 shown to produce cessation of K^+-secretion by the tissue. Vestibular dark cells show many ion transport characteristics similar to the marginal cells of stria vascularis [2], so it is likely that strial marginal cells are similarly influenced. Changes in pH have also been shown to uncouple gap junctions between supporting cells in the organ of Corti [3]. These observations raise the possibility that pH changes of the cochlear fluids could play a role in modulating ion transport processes associated with endolymph homeostasis in the cochlea. One of the goals of the present study was therefore to measure the magnitude of pH changes occurring in the inner ear *in vivo,* under different experimental conditions to establish the primary factors which determine the pH of the cochlear fluids. In addition, we measured the functional consequences of induced pH disturbances.

In similarity with other extracellular fluids, regulation of cochlear fluids pH requires an understanding of the role played by bicarbonate ions in pH buffering [4, 5]. In simplified terms, the pH buffering provided by bicarbonate is based on the equilibrium in which CO_2 and water combine to form one bicarbonate ion and one proton (Equation 1).

$$CO_2 + H_2O \leftrightarrow [H^+] + [HCO_3^-] \quad (1)$$

The pH of a bicarbonate-containing medium thus depends on the partial pressure of CO_2 in the medium. At equilibrium, the relationship between pH, bicarbonate and CO_2 can be calculated as detailed by Hruska [4]. Using a pK for this reaction of 6.1, and calculating the dissolved CO_2 as the product of the partial pressure of CO_2, in mm Hg, multiplied by a solubility constant for CO_2 (estimated to be 0.03 by Hruska [4]), the relationship can be quantified as shown in Equation (2).

$$pH = 6.1 + \log[HCO_3^-]/0.03(pCO_2) \quad (2)$$

Fig. (**1**) illustrates the calculated dependence of pH on CO_2 level for 4 different concentrations of HCO_3^-/CO_2 shown as the percentage concentration, assuming an atmospheric pressure of 760 mmHg. If any one of the three parameters (pH, HCO_3^-, CO_2) is altered, then at least one of the remaining parameters must change until a new equilibrium is established. As a result, the pH of cochlear fluids must depend on the prevailing concentration of CO_2.

In biological systems, the above equilibrium is catalyzed by carbonic anhydrase. Carbonic anhydrase has been demonstrated to be present in high levels in tissues of the cochlea [6]. Using histochemical methods, Lim *et al*. [7] found CA to be localized primarily in the spiral ligament as well as at other sites. Subsequently Spicer and Schulte and Ichimiya *et al*. demonstrated the highest density of CA II in fibrocytes of the lateral wall and limbus [8, 9]. However, the role played by carbonic anhydrase and bicarbonate in cochlear fluids homeostasis is not well established. In the

*Address correspondence to this author at the Department of Otolaryngology, Teikyo University Chiba Medical Center, 3426-3, Anesaki, Ichihara, Chiba, 299-0111, Japan; Tel: +81-436-62-1211;
Fax: +81-436-61-8474; E-mail: suzukima@med.teikyo-u.ac.jp

1874-4281/15 2015 Bentham Open

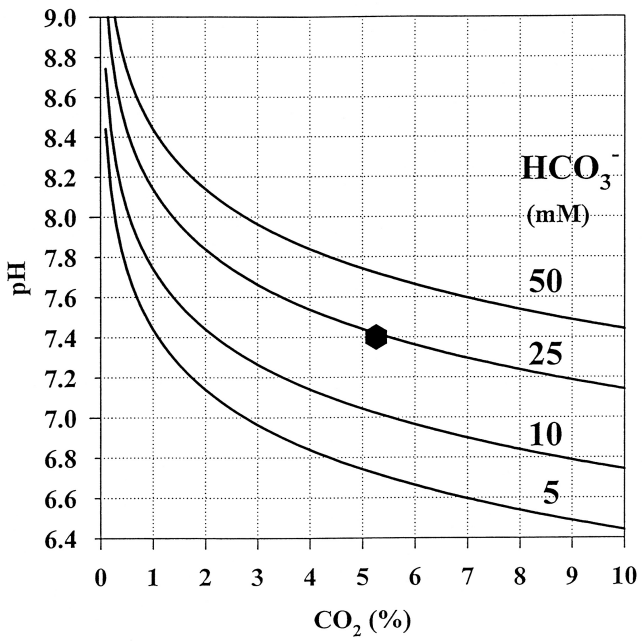

Fig. (1). Relationships between HCO_3^-, pH and CO_2, calculated as described by Hruska [4]. Curves have been calculated for 4 concentrations of HCO_3^-, as indicated. The asterisk indicates the approximate nornal physiological situation, with 25 mM HCO_3^- and 5% CO_2.

present study, we have compared the role played by CO_2 in influencing cochlear fluids pH in the normal cochlea and in the cochlea treated with the carbonic anhydrase inhibitor, acetazolamide (ACZ).

METHODS

This study used 25 pigmented guinea pigs weighing 300-500 g, which were anesthetized with a combination of sodium pentobarbital (25 mg/kg i.p.) and Innovar-Vet (0.35 ml/kg i.m.) (Innovar-Vet contains 0.4 mg/ml fentanyl and 20 mg/ml droperido1). Sodium pentobarbital was supplemented at regular intervals through an intravenous line in the left external jugular vein, as required to maintain deep anesthesia. Body temperature was maintained at 38-39 °C with a thermistor-controlled heating pad. The trachea was cannulated and, prior to recordings, animals were immobilized with gallamine triethiodide (2-3 mg/kg) and artificially ventilated. The head was secured in a rigid head-holder and the right cochlea was exposed by a ventrolateral approach.

The systemic CO_2 level was varied by slightly hyperventilating the animal with room air mixed with a variable amount of CO_2. The end-tidal CO_2 level of expired gas was continuously monitored with a Puritan-Bennett Datex CO_2 analyzer and was used as an index of systemic CO_2. The air/CO_2 mixture was initially adjusted to maintain a physiologic end-tidal level of 5% CO_2 (approximately 38 mmHg partial pressure). Hypercapnea and hypocapnea were subsequently induced by slowly raising or lowering the CO_2 content of the inspired gas using flow meter, while monitoring the end-tidal CO_2 level of expired gas. Hypercapnea or hypocapnea was maintained for a period of 10-20 minutes, then it returned slowly back to the physiologic level. If full recovery was observed, repeated CO_2 manipulations were performed, with a maximum of 6 CO_2 manipulations performed in each animal.

The method and the recording system for the microelectrodes have been described elsewhere [10]. In brief, all measurements of endolymph were made in the second cochlear tum. Electrodes were inserted into the scalae through small (30 to 50 micrometer diameter) fenestras made in the bone with a fine pick after first thinning the bone with a flap knife. The pH of endolymph (pH_e), scala vestibuli perilymph (pH_v) and plasma were measured with double-barreled H^+-selective electrodes. Endolymph K^+, Na^+ and Cl^- concentrations were also measured with double-barreled ion-selective electrodes. For endolymphatic measurements, endocochlear potential (EP) was recorded from the reference barrel of the ion electrode. In some experiments, plasma pH was measured by inserting a double-barreled ion-selective electrode into the right jugular vein.

Double-barreled ion-selective electrodes were made from borosilicate capillary with one barrel silanized by exposure to dimethyldichlorosifane vapor (Sigma, St. Louis). Electrode tips were broken to 2-3 micrometer diameter. For pH electrodes, the ion barrel of the electrodes was filled with 40 mM KH_2PO_4, 23 mM NaOH and 15 mM NaCl [11] and the potential barrel was filled with 500 mM KCI. The exchanger for this electrode was Fluka 95297 (Fluka Chemical, Ronkonkoma, NY, USA), a small column of which was drawn into the ion barrel by suction. For the K-selective electrodes, the ion and potential barrels contained 500 mM KCl and 500 mM NaCl respectively and Fluka 60398 ion exchanger was used. In Na-selective electrodes, the ion and potential barrels contained 500 mM NaCl and 500 mM KCl respectively and Fluka 71732 exchanger was used. For Cl-selective electrodes, the ion barrel contained 500 mM KCl and the potential barrel contained a mixture of 500 mM KCH_3COO and 10 mM KCl. All electrodes were calibrated before and after use in an appropriate range of standard solutions at 38 °C. For pH electrodes, commercially available buffer solutions at pH 8.00, 7.00 and 6.00 were used (Fisher Scientific, Pittsburgh, PA, USA). The standards for K^+ and Cl^- -selective electrodes contained 100, 150 or 200 mM KCl. K^+-selective electrodes were verified to be insensitive to pH changes. Standards for Na^+-selective electrodes contained 1, 2 or 5 mM NaCl in a background of 160 mM KCl. Potentials were recorded from ion electrodes under computer control using digital meters equipped with IEEE-488 interfaces. Readings were typically sampled and stored at 20 sec intervals.

In some experiments, acetazolamide (Sigma, St. Louis, USA) was administered at a dose of 130 mg/kg by an intravenous line in the left jugular vein. Acetazolamide was given as approximately 2 ml of a solution in distilled water (PH 7.35, osmolarity 380 mOsm), given over a 2-3 min period.

Data are presented as the mean and the standard deviation (SD) of the number (n) of observations stated. Unless specified otherwise, statistical significance was determined by analysis of variance with a probability < 0.05 considered significant. All statistical analysis was performed using the Statistical Package for Social Science, version 11.01 (SPSS Inc., Chicago, IL). The study procedures were in accordance with the standards set forth in the eighth

edition of Guide for the Care and Use of Laboratory Animals published by the National Academy of Sciences, The National Academies Press, Washington, DC, USA.

RESULTS

Endocochlear Potential and pH in the Normal State

Pre-treatment values of EP, pH_e and pH_p were measured while respiratory end-tidal CO_2 was maintained at a value close to 5%. Under these conditions, the baseline EP averaged 82.3 mV (SD 3.91, n=19), the pH of endolymph averaged 7.46 (SD 0.09, n=19) and the pH of scala vestibuli perilymph averaged 7.39 (SD 0.15, n=8). Although endolymph pH was often higher than that of perilymph, there was substantial inter-animal variation and the difference was not statistically significant. In addition, using a paired t-test on data from 8 animals in which endolymph and perilymph pH were both measured also did not indicate a significant difference (endolymph 7.45 +/- 0.095; perilymph as above; p= 0.27).

Dependence of pH on Respiratory CO_2

The effects of changes in respiratory CO_2 on the cochlea are illustrated in Fig. (**2**). In this example, the animal was subjected to a period of hypercapnea followed by a period of hypocapnea, during which EP, pH_e and pH_v were monitored simultaneously. Hypercapnea to the point where the end-tidal CO_2 level reached 11.6%, induced a progressive acidification of both endolymph and perilymph. Associated with the acidification was an elevation of the EP above the normal value. Oppositely-directed effects were produced by hypocapnea. Reduction of expired CO_2 to 2.8% produced an alkalinization of endolymph and perilymph and a reduction in the EP. The magnitude and repeatability of induced EP and pH changes for individual experiments are summarized in Fig. (**3**) and mean values are given in Table **1**. The summaries confirm that hypercapnea systematically increases EP and decreases pH_e and pH_v, while hypocapnea decreases EP and increases pH_e and pH_v. All the measured changes in EP, pH_e and pH_v were statistically significant. In Fig. (**3**), pH_e and pH_v changes are shown superimposed with different symbols. For both hypercapnea and hypocapnea, measured changes in pH_e and pH_v were not significantly different (p = 0.270). In addition, in a limited number of experiments plasma pH changes were monitored, which appeared to vary to a greater degree than the pH of the cochlear fluids, but in most animals indicating larger pH changes occurred. Quantifying the dependency of pH and EP on CO_2 is not straightforward, however, as each of these parameters varies in a nonlinear manner as illustrated in Fig. (**4**). In this figure the data from Fig. (**2**) were re-plotted showing the dependence of EP and pH_e on respiratory CO_2 (left two panels) and showing the relationship between EP and pH_e (right panel). Due to some hysteresis of the response, only data points from the initial phase of the hypercapnea and hypocapnea are plotted (data from the recovery phases are not shown). It is apparent that the response of EP and pH_e to CO_2 change is highly nonlinear, with relatively little effect when the CO_2 is in the range of 4% to 10%. Substantially larger changes are induced when the CO_2 levels exceed these amounts. On the other hand the relationship between EP and pH_e was found to be remarkably linear, with a fitted regression line in this case showing a change of -22.4 mV/pH unit increase. The EP response to pH changes averaged across all experiments was -15.61 mV/pH unit (+/- 14.9, n= 23) for hypercapnea and -33.13 mV/pH unit (+/- 30.80, n= 17) for hypocapnea.

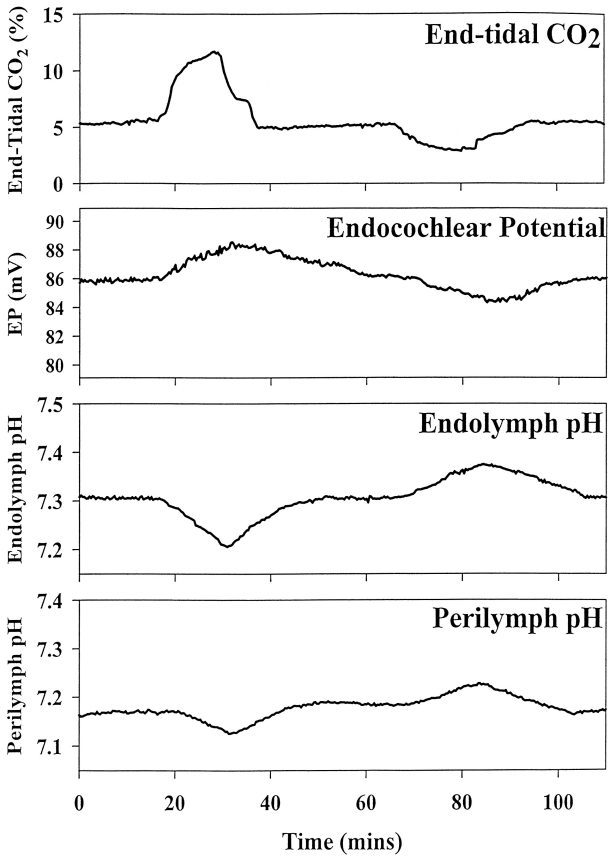

Fig. (2). Example of an experiment in which end-tidal CO_2 was manipulated by varying the CO_2 content of inspired air, while endocochlear potential, endolymph pH and perilymph pH were simultaneously measured. Hypercapnea produced a reversible acidosis of endolymph and perilymph, which was accompanied by an increase in endocochlear potential. Hypocapnea produced oppositely-directed changes.

Effect of Acetazolamide on CO_2-Induced Changes

The effect of intravenous administration of the carbonic anhydrase inhibitor acetazolamide on CO_2-induced pH and EP changes is shown in Fig. (**5**). Acetazolamide caused a decrease in endtidal CO_2, which was subsequently corrected by increasing inspired CO_2, Even before CO_2 was corrected, there was an acidosis of endolymph. Both the decrease in expired CO_2 and the acidosis of endolymph are a consequence of the impairment of CO_2 transport out of the cochlea and other organs. The mean EP decrease with acetazolamide was 7.38 mV (+/- 2.08, n= 12) and the mean endolymph pH decrease was 0.26 (+/- 0.04, n= 5). The mean induced changes for K^+_e, Na^+_e and Cl^-_e were 8.26 mM (+/- 3.98, n= 3), 0 (n= 2) and 0.43(+/- 0.66, n= 3), respectively. Only the decrease in K^+_e was statistically significant.

Hypercapnea in the presence of acetazolamide did not induce EP increases or pH decreases of comparable magnitude to those seen in the untreated state. With an average induced CO_2 increase to 10.62%, the mean EP change was -0.08 mV (+/- 0.86, n= 13) and the mean pH_e

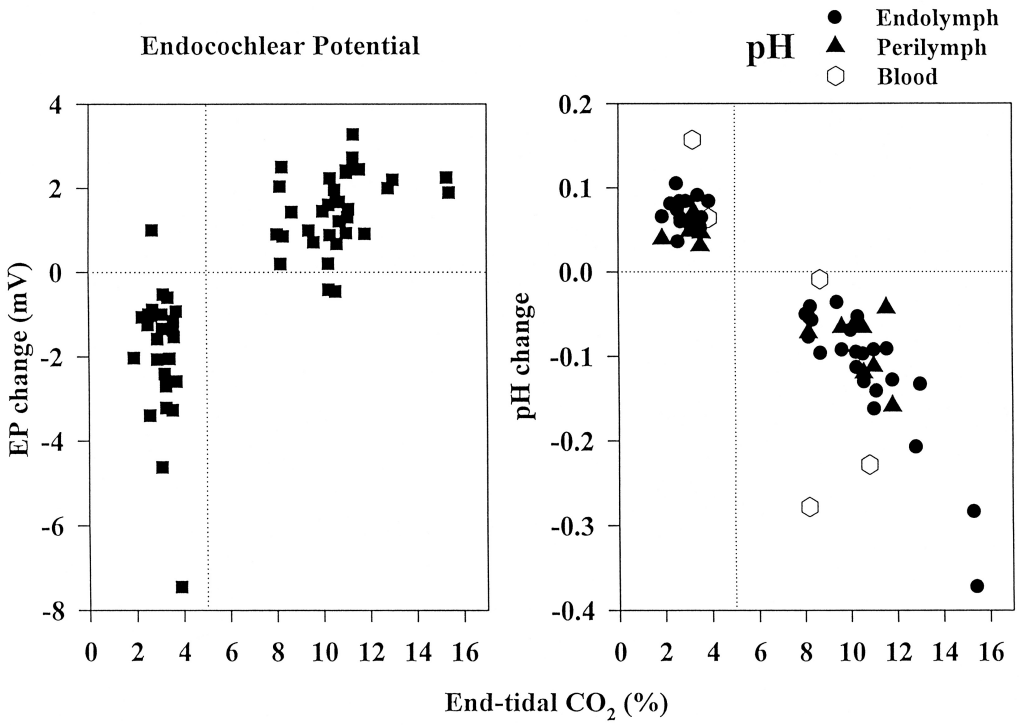

Fig. (3). Summary of the peak endocochlear potential changes (left) and fluids pH changes (right) occurring during hypercapnea and hypocapnea. In addition, a limited number of measurements of venous blood pH are shown. Hypercapnea induces a systematic EP increase and pH decrease, while hypocapnea induces oppositely directed effects.

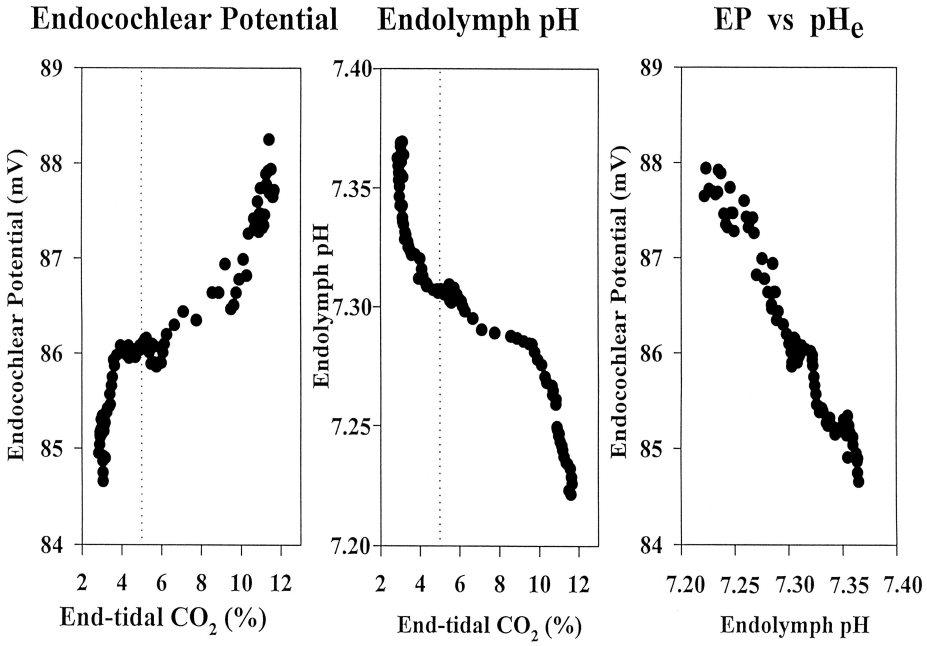

Fig. (4). Dependence of endocochlear potential (left) and endolymph pH changes (center) with end-tidal CO_2 level during the onset of hypercapnea and hypocapnea. The relationship is nonlinear, with greater pH changes occurring when CO_2 is outside the range of approximately 4% to 9%. In comparison, the endocochlear potential shows an approximately linear dependence on endolymph pH (right).

change was -0.03 mV (+/- 0.03, n= 6), which were both significantly smaller than observed in the untreated state.

Changes of K^+, Na^+ and Cl^- Induced by CO_2 Manipulations

The possible occurrence of changes in other endolymph electrolytes was also investigated. Fig. (**6**) illustrates the effect of CO_2 manipulations before and after ACZ treatment on K_e and EP. Associated with the CO_2-induced EP changes we observed systematic changes in K^+_e which was found to increase as EP decreased and to decrease as EP increased. The K^+_e changes were statistically significant but neither Cl^-_e nor Na^+_e showed significant concentration change (Table **2**).

Table 1. EP and pH changes induced by hypercapnia and hypocapnea.

	Hypercapnea	Hypocapnea
CO_2 level	+10.64% ± 1.7 (n=34)	3.08% ± 0.49 (n=26)
EP change	+1.46 mV ± 0.87 (n=34)	-1.92 mV ± 1.60 (n=26)
Endolymph pH change (pH_e)	-0.12 ± 0.08 (n=23)	0.07 ± 0.02 (n=17)
Perilymph pH change (pH_v)	-0.08 ± 0.04 (n=9)	0.05 ± 0.02 (n=6)

CO_2-induced K^+_e changes became insignificant after treatment with ACZ (Table **2**).

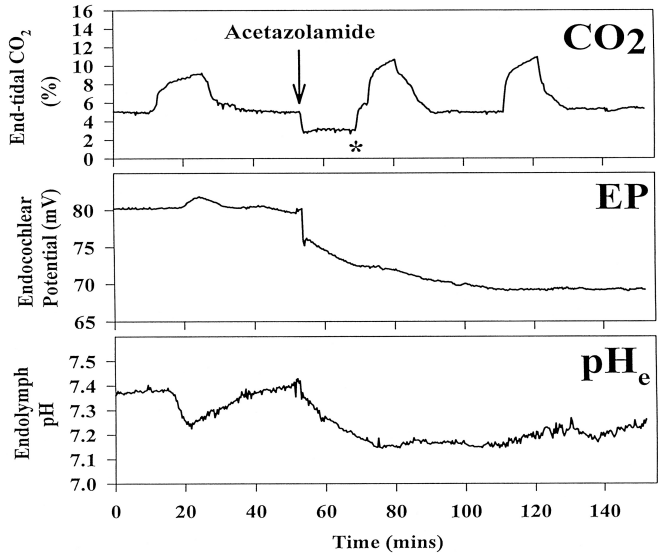

Fig. (5). Dependence of CO_2-induced endocochlear potential (EP) and endolymph pH (pH_e) changes on acetazolamide (ACZ). Administration of 130 mg/kg acetazolamide reduces expired CO_2 until the inspired level was adjusted (*) and reduces EP and pH_e. It also abolishes the EP increases and pH_e decreases associated with hypercapnea.

DISCUSSION

Investigation of pH regulation in the cochlea, and the influence of pH changes on ion transport systems, is made complex by the interactions of CO_2 and HCO_3^- with pH. The present study demonstrates the strong influence of vascular CO_2 on the pH of cochlear fluids, and on the magnitude of the EP and on K^+_e. These effects are presumed to be the result of CO_2 changes of the cochlear fluids which parallel the induced vascular change. They demonstrate that endolymph and perilymph pH are not closely regulated as independent compartments, but instead are markedly affected by systemic variations in CO_2. These observations have relevance to the wide range of experimental studies performed on the ear, since they emphasize the need to maintain the respiratory CO_2 level stable and within the normal range to maintain the normal physiologic state. Spontaneously respiring, anesthetized guinea pigs commonly show an elevation of expired CO_2 especially when deeply anesthetized. Furthermore, for experimental animals which are artificially ventilated but without CO_2 monitoring, it is our experience that under-ventilation is apparent by cyanosis, but over-ventilation is not readily apparent. In order to maintain CO_2 constant and thus maintain cochlear fluid pH and EP in a stable state in anesthetized animals, artificial ventilation with CO_2 monitoring would appear necessary.

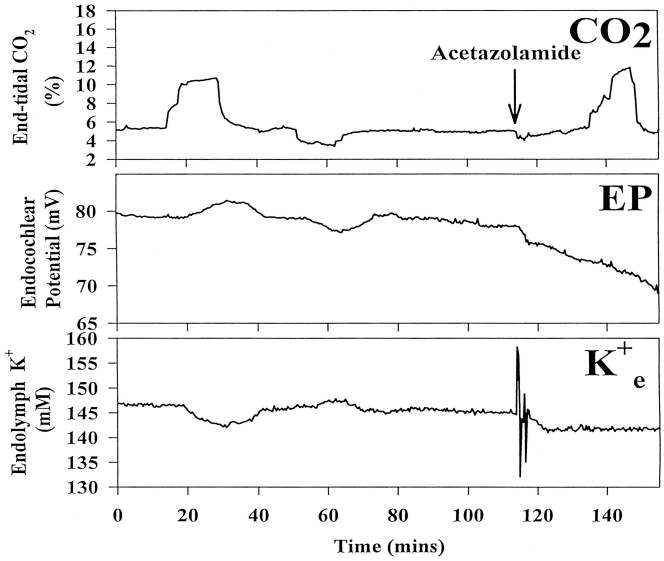

Fig. (6). The effect of CO_2 manipulations before and after acetazolamide (ACZ) treatment on EP and endolymph K^+ (K_e).

The pH of endolymph measured in this study by ion-selective electrodes is comparable to that measured in prior studies [12, 13]. The mean endolymph pH was found to be slightly higher than that of perilymph. Paired measurements made in the same animal did not demonstrate a significant difference, but the number of animals was limited. Nevertheless, a slightly higher endolymph pH would be consistent with the higher bicarbonate level measured in endolymph by Ikeda *et al*., who found the HCO_3^- concentration to be 23 mM in perilymph and 40.0 mM in endolymph [14].

The observation that EP is increased by elevation of respiratory CO_2 confirms similar observations by Prazma *et al*., in studies which demonstrated that EP increases were produced when animals were respired with 90% O_2 + 10% CO_2 [15]. In the present study, we found similar increases of EP by CO_2 elevation without changing respiratory O_2. One explanation is that the EP increase could result from a CO_2-induced increase in cochlear blood flow, as documented by Hultcrantz *et al*. [16]. In the latter study they found that ventilation with 10% CO_2 in air produced a 27% increase in cochlear blood flow as measured by Laser Doppler flowmetry. It is also possible, however, that the induced pH

Table 2. Ion changes induced by hypercapnea and hypocapnea.

	Hypercapnea	Hypocapnea	Hypercapnea ACZ-Treated
K (mM)	-2.86 ± 1.17 (n=4)	2.88 ± 2.43 (n=3)	-0.79 ± 1.46 (n=3)
Na (mM)	0.017 ± 0.016 (n=3)	-0.013 (n=2)	0.003 (n=2)
Cl (mM)	-0.63 ± 0.39 (n=4)	-0.21 ± 0.42 (n=4)	-0.12 ± 0.25 (n=3)

changes of the cochlear fluids affect ion transport more directly, consistent with the acid-induced increase in potential shown to be generated *in vitro* across vestibular dark cells by Wangemann *et al*. [2]. The almost linear relationship between EP and pH would appear more in keeping with the modulation of an ion transport process. However, a determination of the possible contributions of the different tissues involved is beyond the scope of this study, and many other cell types, including basal cells, fibrocytes, outer sulcus cells, cells of the organ of Corti and Reissner's membrane, etc., could possibly contribute to the observed changes.

The comparison of EP and K^+_e changes induced by CO_2 manipulations demonstrates a negative correlation, in which an increase of EP was associated with a decrease of K^+_e and vice versa. This result is difficult to explain by pH modulating K transport into endolymph, as an increase in K transport would be expected to increase both parameters. Other factors therefore need to be considered to explain these findings. One issue is the consideration of electroneutrality, in which any increase or decrease in K must be balanced of influx or efflux of other ions so that numbers of anions and cations remain equal. At the time when K^+_e decreased, no increase in Na^+_e was observed, suggesting that the anion concentration of endolymph must also be changed. Since no Cl^-_e change was detected, it is most likely that endolymph HCO_3^- concentration (not measured) was decreased. Little is known about the mechanisms underlying endolymph HCO_3^- homeostasis. It would be expected that HCO_3^- would enter endolymph passively through channels such as the CFTR (cystic fibrosis transmembrane conductance regulator) [17] or other HCO_3^--permeable anion channel, driven by the large electrochemical gradient dominated by the EP. HCO_3^--removal from endolymph is likely to parallel the process shown to occur in the kidney tubule, accomplished predominantly by acid secretion into the lumen. The secreted acid neutralizes HCO_3^-, forming CO_2 and water. In the cochlea, the released CO_2 would readily diffuse across the tissue boundaries out of the endolymphatic space. Such a scheme is supported by anatomic studies which have shown that the interdental cells of the cochlea show a distribution of acid transporting proteins comparable to the acid-secreting intercalated cells of the kidney [18]. The same study showed that Cl^-/HCO_3^--exchanger was associated with basolateral membranes of cells bounding endolymph and is hence unlikely to be directly involved in removing HCO_3^- from endolymph. If the systemic pH reduction reduced the rate of HCO_3^- entry or increased the rate of acid secretion into endolymph, either of these scenarios would result in an increase of EP, a decrease in pH, a decrease of endolymph HCO_3^- and a resulting decrease of K^+_e. While such a hypothesis appears to account for the experimental findings, there are still other processes which could play a part. One example is the fact that the above explanation assumes that observed ion concentration changes reflect the total amount of the ion in endolymph and assumes that the total endolymph volume remains unchanged. Alternatively, the reduction of K^+ and HCO_3^- could result from water entry diluting unchanged quantities of endolymph solutes. Even if a reduction in total endolymph K^+ and HCO_3^- occurred, the osmotic change could result in a decrease in endolymph volume. In this case, the total ion change could be underestimated by endolymph concentration measurements. In the present study, the stability of Cl^- and Na^+ may argue against the occurrence of significant volume changes. However, a small possibility exists that a complex combination of Cl^-, K^+, HCO_3^- and volume changes could all be occurring.

In studies where the vascular systems of isolated cochleae were perfused with artificial media [19], it was found that reduction of CO_2 caused a reduction of EP, a finding which is in agreement with the observations of the present *in vivo* study. CO_2 elevations well above those employed in the present study were also evaluated in their *in vitro* preparation, which were also found to induce a reduction in EP. CO_2 elevations in the range reported here (between 5 and 15%) were not evaluated in their study.

Systemic administration of ACZ reduced EP, pH_e and K^+. The EP and pH reductions observed are comparable to those seen in prior studies after ACZ treatment [14, 20, 21], yet differ from a study which used methazolamide as the inhibitor, in which an EP increase was observed [15]. The EP decrease cannot be a secondary consequence of the pH decrease and presumed CO_2 accumulation in the cochlea, as such changes have been shown in this study to result in EP increases, rather than decreases. We did not detect any change in Cl^-_e, which is in agreement with Ikeda *et al*., [14] but differs from the Cl^-_e reduction observed by Sterkers *et al*. [21]. In addition, our finding of a K^+_e reduction also differed from the Sterkers study in which no K^+_e change was found. The differences found between the present study and, Sterkers' study may be accounted for by the different species (rat) involved, the different time courses (fluids sampled at least two hours after ACZ administration), or the fact that animals were allowed to spontaneously respire, rather than being artificially ventilated, so that systemic CO_2 levels may have differed. Following systemic administration of ACZ, CO_2 manipulations induced markedly smaller EP, pH and K^+_e changes. A reduction in CO_2-induced EP changes was also reported by Prazma [15]. Our findings confirm prior reports, demonstrating the involvement of carbonic anhydrase in EP generation, pH regulation and in the response to CO_2 disturbance. The observation that both EP and K^+_e are reduced by ACZ, combined with the observation by Ikeda *et al.* [14], that endolymph HCO_3^- is also reduced,

would be consistent with a decrease in K transport into endolymph. This could possibly be the result of disturbance in K^+ movements through the fibrocytes of the lateral wall. The fibrocytes are known to be rich in carbonic anhydrase, but the functional role of carbonic anhydrase, HCO_3^- and/or H^+ remains to be determined. Finally, the ACZ-induced reduction in K^+_e is unlikely to be secondary to the reduction in endolymph HCO_3^-, as a reduced entry of HCO_3^- into endolymph would be expected to increase EP.

The role of pH and HCO_3^- in endolymph homeostasis continues to remain a highly complex issue. As HCO_3^- represents the third-most prominent ion in endolymph, it undoubtedly plays a significant role in endolymph homeostasis. An understanding of how endolymph composition and volume is maintained thus requires a more detailed knowledge of the all relevant transport processes and the mechanisms by which each is regulated.

CONFLICT OF INTEREST

This was not an industry-supported study. Authors have no conflict of interest to declare in relation to the subject matter of this manuscript.

ACKNWLEDGEMENTS

Authors would like to express their appreciation for Dr. Alec N. Salt, Department of Otolaryngology, Washington University in St. Louis, MO, USA, for his guidance.

REFERENCES

[1] Wangemann P. Comparison of ion transport mechanisms between vestibular dark cells and strial marginal cells. Hear Res 1995; 90: 149-57.

[2] Wangemann P, Liu J, Shiga N. The pH-sensitivity of transepithelial K^+ transport in vestibular dark cells. J Membrane Biol 1995; 147: 255-62.

[3] Sato Y, Santos-Sacchi J. Cell coupling in the supporting cells of Corti's organ: sensitivity to intracellular H^+ and Ca^{2+}. Hear Res 1994; 80: 21-4.

[4] Hruska K. Pathophysiology of acid-base metabolism. In: Klahr S, Ed. The kidney and body fluids in health and disease. New York: Plenum Press 1983; pp. 223-67.

[5] Maren TH. The kinetics of HCO_3^-synthesis related to fluid secretion, pH control and CO_2 elimination. Ann Rev Physiol 1988; 50: 695-717.

[6] Erulkar SC, Maren TH. Carbonic anhydrase and the inner ear. Nature 1961; 189: 459-60.

[7] Lim DJ, Karabinas C, Trune DR. Histochemical localization of carbonic anhydrase in the inner ear. Am J Otolaryngol 1983; 4: 33-42.

[8] Spicer SS, Sculte BA. Differentiation of inner ear fibrocytes according to their ion transport related activity. Hear Res 1991; 56: 53-64.

[9] Ichimiya I, Adams JC, Kimura RS. Immunolocalization of Na^+, K^+-ATPase, Ca^{++}ATPase, calcium-binding proteins, and carbonic anhydrase in the guinea pig inner ear. Acta Otolaryngol 1994; 114: 167-76.

[10] Suzuki M, Kikuchi T, Ikeda K. Endocochlear potential and endolymphatic K changes induced by gap junction blockers. Acta Otolaryngol 2004; 124: 902-6.

[11] Ammann D, Lanter F, Steiner RA, Schulthess P, Shijo Y, Simon W. Neutral carrier based hydrogen ion selective microelectrode for extra-and intracellular studies. Anal Chern 1981; 53: 2267-9.

[12] Misrahy GA, Hildreth KM, Clark LC, Shinabarger EW. Measurement of the pH of the endolymph in the cochlea of guinea pigs. Am J Physiol 1958; 194: 393-5.

[13] Bosher SK. The nature of the negative endocochlear potentials produced by anoxia and ethacrynic acid in the rat and guinea pig. J Physiol (Lond) 1979; 293: 329-45.

[14] Ikeda K, Kusakari J, Takasaka T, Saito Y. Early effects of acetazolamide on ionic activities of the guinea pig endolymph: evidence for active function of carbonic anhydrase in the cochlea. Hear Res 1987; 31: 211-6.

[15] Prazma J. Carbonic anhydrase in the generation of cochlear potentials. Am J Physiol 1978; 235: F317-20.

[16] Hultcrantz E, Larsen HC, Angelborg C. The effects of CO_2 -breathing on cochlear blood flow. Arch Otorhinolaryngol 1980; 228: 211-5.

[17] Gray MA, Plant S, Argent BE. cAMP-regulated whole cell chloride currents in pancreatic duct cells. Am J Physiol 1993; 264: C591-602.

[18] Stankoic KM, Brown D, Alper SL, Adams JC. Localization of pH regulating proteins H^+ATPase and Cl^-/HCO_3^-exchanger in the guinea pig inner ear. Hear Res 1997; 114: 21-34.

[19] Arakawa E, Marcus DC, Thalmann R. Dependence of endocochlear potential on vascular pH. Hear Res 1987; 31: 1-7.

[20] Kuijpers W, Bonting SL. The cochlear potentials II. The nature of the endolymphatic resting potential. Pflugers Arch 1970; 320: 359-72.

[21] Sterkers O, Sawnon G, Tran Ba Huy P, Ferrary E, Amie, C. Electrochemical heterogeneity of the cochlear endolymph: effect of acetazolamide. Am J Physiol 1984; 246: F47-53.

Received: December 1, 2014 Revised: January 7, 2015 Accepted: January 22, 2015

© Suzuki *et al.*; Licensee *Bentham Open*.

This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.