EQUILIBRIUM CONDITIONS FOR CALCIUM – CARBONATE OTOCONIA IN ACCELERATION – SENSITIVE RECEPTOR ORGANS SUCH AS THE BULLFROG'S SACCULUS

The aural receptor organs sensitive to linear acceleration are characteristically filled with calcium-carbonate intertial masses. These may be solid structures, such as piscine otoliths, or clusters of fine grains, such as amphibian, reptilian, avian, or mammalian otoconia. The mineral form may be aragonite (as in the frog's sacculus), calcite, vaterite, or calcium carbonate monohydrate (Kurc *et al*., 1999). In every instance, however, the mineral is likely to be in equilibrium with soluble Ca^{2+}, bicarbonate ion, and carbonate ion at the prevailing pH and pCO_2. These notes are meant to explore the restrictions that this condition places upon the free Ca^{2+} concentration in endolymph and the ways in which this concentration may be estimated.

Equilibrium conditions

The pK_as for the two dissociation steps of carbonic acid (p. **8**-43, Lide, 1996) are:

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \qquad pK_{a1} = 6.35 \qquad K_{a1} = 4.47\cdot10^{-7}$$

$$HCO_3^- \rightleftharpoons H^+ + CO_3^{2-} \qquad pK_{a2} = 10.33 \qquad K_{a2} = 4.68\cdot10^{-11}$$

For the first relation,

$$K_{a1} = \frac{\left[H^+\right]\left[HCO_3^-\right]}{\left[H_2CO_3\right]}.$$

The concentration of bicarbonate in plasma is ordinarily not measured directly; instead, because the ion is in rapid equilibrium with dissolved CO_2 as a result of carbonic anhydrase activity, the pH and P_{CO2} are measured with glass electrodes and the bicarbonate concentration is inferred from the gas's solubility, α. It follows that

$$\left[H^+\right] = \frac{K_{a1}\left[H_2CO_3\right]}{\left[HCO_3^-\right]} = \frac{K_{a1}'\alpha P_{CO_2}}{\left[HCO_3^-\right]}.$$

The modified dissociation constant, which corresponds to $7.94\cdot10^{-7}$, stems from the use of P_{CO2} as a surrogate for the carbonic-acid concentration. The relation yields the modified Henderson-Hasselbalch equation

$$pH = p\,K_{a1}' + \log\left\{\frac{\left[HCO_3^-\right]}{\alpha P_{CO_2}}\right\}.$$

For normal bullfrogs (*Rana catesbeiana*) at 25° C, the arterial plasma pH = 7.87, $P_{CO2} = 1.72$ kPa, $\alpha = 305.3$ nM·Pa^{-1}, $pK_{a1}' = 6.137$ ($K_{a1}' = 7.29\cdot10^{-7}$), and the calculated bicarbonate concentration is 28.4 mM (Toews and Stiffler, 1990). In another determination at 25° C (Tazawa *et al*., 1979), the averaged arterial pH = 7.84 and $P_{CO2} = 14$ torr = 1.87 kPa; using the pK_{a1}' and α values above, the bicarbonate concentration is 28.8 mM. At 15° C, the arterial plasma pH = 7.90, $P_{CO2} = 10.6$ torr = 1.41 kPa, $pK_{a1}' = 6.189$ (from turtles; $K_{a1}' = 6.47\cdot10^{-7}$), and the calculated bicarbonate concentration is 26.8 mM (averaged control points from Figures 1 and 2, Warburton *et al*., 1989).

From the values above, it then follows that

$$\left[CO_3^{2-}\right] = \frac{K_{a2}\left[HCO_3^-\right]}{\left[H^+\right]} = 93.2\ \mu M - 99.6\ \mu M \approx 97\ \mu M.$$

The blood pH varies only slightly around our normal experimental temperature of 21° C; for bullfrogs (Malan *et al*., 1976), the blood pH = $8.184 - 0.0206 \cdot T$ (7.75 at 21° C). Note that for skeletal muscle, the intracellular pH = $7.275 - 0.0152 \cdot T$ (6.96 at 21° C); internal solutions for tight-seal, whole-cell recording should be made essentially neutral.

For $CaCO_3$ as calcite, the solubility product $K_{SP} = [Ca^{2+}] \cdot [CO_3^{2-}] = 3.36 \cdot 10^{-9}$ (p. **8**-91, Lide, 1996). In a saturated solution at equilibrium,

$$\left[Ca^{2+}\right] = \frac{K_{SP}}{\left[CO_3^{2-}\right]} = 33.7\ \mu M - 36.1\ \mu M \approx 35\ \mu M.$$

The relations above can be combined to emphasize the strong dependence of the equilibrium Ca^{2+} concentration on pH:

$$\left[Ca^{2+}\right] = \frac{K_{SP}\left[H^+\right]^2}{K'_{a1}K_{a2}\alpha P_{CO_2}} \approx \frac{7.33 \cdot 10^{10}}{10^{(2 \cdot pH)}}.$$

Our group's preliminary measurements with ion-selective microelectrodes indicated that the free Ca^{2+} concentration in bulk saccular endolymph is about 260 μM (Corey and Hudspeth, 1983). If this value and the calculations are correct, it follows that the endolymphatic pH differs from that in plasma or that the ions are not at equilibrium.

Measurement of the extracellular Ca^{2+} concentration

There are potentially two aspects to the endolymphatic Ca^{2+} concentration of the frog's sacculus: the bulk concentration throughout the otoconial mass, and the local concentration at the site of mechanoelectrical transduction. The former value, which presumably reflects the ionic equilibrium discussed above, may be measured with ion-selective electrodes. Alternative measurement approaches involve atomic-emission spectrophotometry or electron-probe microanalysis (Peterson *et al*., 1978), which have the disadvantage of measuring the total Ca^{2+} concentration rather than only the free component.

Because of active Ca^{2+} extrusion by stereociliary Ca^{2+}-ATPase, the concentration of free Ca^{2+} around saccular hair bundles may be substantially elevated – perhaps doubled – with respect to the bulk endolymphatic concentration (Yamoah *et al*., 1998). The effect of pumping could be further investigated with the technique employed in that study, the vibrating, self-referencing Ca^{2+} electrode. Because this approach requires that the electrode move into and out of the space of interest, however, it is unlikely to leave a standing Ca^{2+}-concentration gradient intact. Another possible technique for measuring the Ca^{2+} concentration near stereocilia would involve confocal microscopy and Ca^{2+}-sensitive indicators. For an objective lens of high numerical aperture and at a high magnification, conventional confocal sectioning permits the analysis of an ovoid volume about 0.9 μm long and 0.4 μm wide, and thus small by comparison with the dimensions of a hair bundle. Moreover, because observations could in principle be conducted across the thickness of the saccular macula (at least near its edge), the technique might be made to operate with an essentially intact sacculus with a functional blood supply. The only necessary penetration of the organ would be that to supply the Ca^{2+} indicator, which might be intoduced as a solid to minimize perturbation of the native endolymph.

The optimal Ca^{2+} indicator would have a dissociation constant near the Ca^{2+}-concentration range of interest. The nearest approximations at present are Fluo-4FF (K_D=40 μM; Molecular Probes F-23980) and Fluo-5N (90 μM; F-14203). If its background (zero-Ca^{2+}) fluorescence is similar to that of other fluo indicators, about 10% of the peak value, the latter compound would be expected to display a modulation in its fluorescence of about 9% (77% to 86%) between Ca^{2+} concentrations of 260 μM and 500 μM. Especially because the required temporal resolution would be relatively low, of the order of seconds, such a signal should suffice for an estimate of any standing Ca^{2+}-concentration gradient.

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