pH Regulation in Tissue-Cultured Bovine Lens Epithelial Cells

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Summary. The intracellular pH (pHi) of tissue-cultured bovine lens epithelial cells was measured in small groups of 6 to 10 cells using the trapped fluorescent dye 2',7'-bis-(2-carboxyethyl)-5 (and 6)carboxyfluorescein (BCECF). When perifused at 35°C with artificial aqueous humour solution (AAH) containing 16 mM HCO₃ and 5% CO₂, pH 7.25, pHi was 7.19 ± 0.02 (SEM, n = 95). On removing HCO₃ and CO₂, there was an initial transient alkalinization followed by a fall in pH to a steady value of 6.97 ± 0.03 (SEM, n = 54). Addition of 0.25 mM 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS) to AAH containing HCO₃ and CO₂ led to a rapid and pronounced fall in pH. Exposure to Na⁺-free AAH again led to a marked fall in pH, but in this case the addition of DIDS did not produce a further fall. Substitution of the impermeant anion gluconate for CI⁻ in the presence of HCO₃ led to a rise in pHi, while substitution in the absence of HCO₃ led to a fall in pHi. The above data indicate a significant role for a sodium-dependent CI⁻-HCO₃⁻ exchange mechanism in the regulation of pHi. Addition of 1 mM amiloride to control AAH in both the presence and absence of HCO₃ led to a marked fall in pHi, indicating that a Na⁺/H⁺ exchange mechanism also has a significant role in the regulation of pHi. There is evidence for a lactic acid transport mechanism in bovine lens cells, as addition of lactate to the external medium produced a rapid fall in pH. Larger changes in pHi were observed in control compared to HCO₃-free AAH and in the latter case a pronounced alkalinizing overshoot was obtained on removing external lactate. Tissue-cultured bovine lens cells thus possess at least three membrane transport mechanisms that are involved in pH regulation. The buffering capacity of the lens cells was measured by perturbing pH with either NH₄⁺ or procaine. The values obtained were similar in both cases and the intrinsic buffering capacity measured in the absence of external HCO₃⁻ was 5 mEq/pH unit (procaine). However, in the presence of HCO₃⁻ and CO₂ the buffer capacity increases approximately fourfold, indicating that HCO₃⁻ is the principal intracellular buffer.

Key Words: Lens pH · pH regulation · bicarbonate · BCECF · CI⁻-HCO₃⁻ · exchanger

Introduction

The lens must continually and actively extrude protons for two main reasons. Firstly, in common with most cells, the lens has a relatively high negative resting potential and thus there is an inwardly-directed electrical gradient for mobile positive charge. Secondly, as metabolism is largely anaerobic, there is a continuous production of H⁺ from lactic acid.

Earlier studies on the rat lens using ion-sensitive microelectrodes showed that the pH in the outermost cells was in the region of 7.0 (Bassnett & Duncan, 1986) and hence the lens must actively extrude H⁺. Dynamic studies of pH regulation in the whole lens are limited by the syncitial nature of the lens and long diffusion paths from the external surface. However, epithelial monolayer (Bassnett, 1990) and tissue culture techniques (Stewart et al., 1988) have been developed to overcome this problem.

Most of the previous studies have been carried out either on the amphibian (Wolosin, Alvarez & Candia, 1988) or the chick lens epithelial system (Bassnett, 1990) using the powerful and convenient fluorimetric dye assay technique. For example, Wolosin et al. (1988) showed that Na⁺-H⁺ exchange was the most important regulatory mechanism in the amphibian lens and, furthermore, that this was activated by a change in lens volume. Bassnett (1990) confirmed both of these findings in the isolated chick lens epithelium but pointed out that CI⁻-HCO₃⁻ exchange also had a role to play. In the toad lens epithelial cells, however, Wolosin, Alvarez & Candia (1989) could find no evidence of CI⁻-HCO₃⁻ exchange, although they suggested that a Na⁺-HCO₃⁻ cotransporter was present in their system.

In the present study fluorimetric assay techniques were applied to mammalian lens cells. In particular the role of sodium-dependent CI⁻-HCO₃⁻ exchange in pH regulation was investigated since a SITS inhibitable, Na⁺-dependent increase in ³⁶Cl⁻ efflux has been shown to occur in the mammalian lens in response to an acid stress (Duncan et al. 1992). A possible role for a lactate-transporting mechanism in lens cell pH regulation was also inves-
tigated as Bonanno (1990) has reported the presence of such a system in rabbit corneal epithelial cells.

Materials and Methods

Tissue Culture

Whole bovine eyes were obtained from a local abattoir and the lens dissected out within 4–6 hr of death of the animal. The epithelium was then placed in a 35-mm plastic culture dish and covered with 2 ml of Eagle’s Minimal Essential Medium (EMEM) (10% fetal calf serum (FCS)) to initiate the primary explant culture. Confluent cultures were obtained within 2–3 weeks. The cells were then trypsinized and subcultured by placing a drop of medium containing 2 × 10⁴ cells on the center of a clean plastic dish and leaving it to settle for 24 hr. A further 2 ml of EMEM were added and the cells allowed to proliferate for 3 days. At the end of this period the cells were confluent and ready for use.

Experimental Solutions

In order to make changes in the concentrations of external Na⁺, Cl⁻ and HCO₃⁻, the cells were equilibrated in a relatively simple artificial aqueous humour (AAH) solution for at least 30 min before the start of any experimental protocol. Control AAH had the following composition (in mM): 124 NaCl, 5 KCl, 0.5 MgCl₂, 1 CaCl₂, 10 HEPES (N-(2-hydroxyethyl)piperazine-N’-(2-ethanesulfonic acid)), 5 glucose and 16 NaHCO₃. This solution was gassed with 5% CO₂/95% O₂ and adjusted to pH 7.25.

Sodium-free solutions contained 124 mM N-methyl-D-glucamine and 16 mM choline bicarbonate, and the pH was set to 7.25 by adding HCl. Chloride-free solutions contained equivalent amounts of D-gluconate in place of chloride.

Bicarbonate-free solutions were ungassed and 16 mM NaHCO₃ was replaced by 16 mM NaCl in the control solution, by 16 mM N-methyl-D-glucamine chloride in the Na⁺-free experiments and by 16 mM sodium gluconate in the Cl⁻-free experiments.

In experiments where the effects of L(+)-lactate, procaine and ammonium chloride were studied, equimolar amounts of NaCl were omitted to accommodate the test substance in each case. All solutions were equilibrated, and pH was adjusted at 35°C.

Dye Loading and Fluorescence Measurements

On removal from the CO₂ incubator (35°C), the tissue-cultured cells were washed twice with 4 ml control AAH and then incubated for 30 min in 4 ml control AAH to which 5 μM of the fluorescent dye 2',7'-bis (carboxyethyl)-5,6-carboxyfluorescein acetoxymethyl ester (BCECFam) had been added. The cells were finally incubated in fresh AAH for at least 30 min in the presence or absence of HCO₃⁻ (depending on the experimental protocol) to permit complete deesterification of the dye to the membrane impermeant BCECF form.

A plastic insert was then sealed into the culture dish with inlet and outlet ports to allow rapid perfusion of the central region of cells (approximately 1 cm²). The time for solution turnover in the central chamber was approximately 1 sec. The experimental solutions were prewarmed before entering the chamber mounted on the heated stage of an inverted microscope (Zeiss IM35), and the cells were maintained at a constant temperature of 35°C. The central cells were irradiated alternatively with 495 and 440 nm light and fluorescence from the trapped dye was measured at 510 nm. The necessary monochromators, dichroic filters and photometers to produce and detect the fluorescence from approximately 6 to 10 cells in the field of view were part of a Photon Technology International (PTI) Deltascan system, and the software systems to control the monochromators and both acquire and process the data were also supplied by PTI.

At least four replicate experiments of each protocol were carried out on separate dishes of cells. At the end of each experiment the cells were perfused with 10 μM nigericin dissolved in a high potassium (150 mM) buffer. Nigericin is a K⁺/H⁺ exchanger and equilibrates the internal pH with the known external pH. The pH standard contained 10 mM piperazine-N,N’-bis(2-ethanesulfonic acid) (PIPES) at pH 6.5, otherwise all standards contained 10 mM HEPES. As the response ratio was linear in the range 7.5 to 6.5, a simple transformation was used to calculate the corresponding pH values from the ratios (Fig. 1). pH values outside this range should be taken as approximations, but in fact most of the experimental protocols gave values within the linear range. Where appropriate the results are expressed as mean ± SEM (number of dishes).

Results

The resting pH of tissue-cultured bovine lens epithelial cells was found to be very sensitive to alterations in external CO₂ and HCO₃⁻. For example, when CO₂ and HCO₃⁻ are removed from the medium, there is a rapid alkalization of pHᵢ, presumably corresponding to a rapid loss of CO₂, and this is followed by a slower acidifying phase probably due to the loss of internal HCO₃⁻ (Fig. 2). On return to control medium, the reverse occurs. Due to these transient changes in internal pH, in order to investigate the effect of CO₂ and HCO₃⁻ on the resting pH, it was necessary to pre-equilibrate the cells in the appro-