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Carbonic anhydrase content of turtle urinary bladder mucosal cells

In the isolated urinary bladder of the fresh water turtle (*Pseudemys scripta*), both the active acidification of the mucosal fluid[1,2] and the active transport of Cl^- (ref. 3) are inhibited by Diamox[4]. Attempts to relate these effects to inhibition of carbonic anhydrase (EC 4.2.1.1) were unsuccessful because of the failure to demonstrate the presence of the enzyme in this tissue[5]. A similar problem, *i.e.* the sulfonamide-inhibitable active transport of Cl^- by a tissue lacking in detectable carbonic anhydrase, exists in the frog cornea[6]. Because carbonic anhydrase may play a role in the active transport of anions by epithelial tissues, we have reexamined the epithelial mucosa of the turtle bladder and have detected the enzyme activity in homogenates of mucosal cells.

Pseudemys scripta were obtained in February and March from Lemberger Co., Oshkosh, Wisc. The entire procedure was designed to minimize the possibility of contaminating the mucosal cell preparation with carbonic anhydrase-rich erythrocytes. The urinary bladders were carefully dissected from exsanguinated turtles, the serosal capillaries drained by gentle pressure, and the tissue rinsed with Ringer's solution. The necks of 6–8 bladders, in the form of closed sacs, were each tied to the outlet of a Luer lock syringe, filled with and immersed in Ca^{2+}-free Ringer's solution containing 17 mM HCO_3^- and 2 mM EDTA, and incubated for 40–60 min at 25°. The fluid containing the mucosal cells was collected from each bladder and separately centrifuged at 5000 × *g* for 15 min. There was no visible trace of erythrocyte contamination in the pellets from any of the bladders. The mucosal cell pellets from the bladders were pooled, weighed, suspended in approx. 3 vol. of distilled water and sonicated with a Branson sonifier for 1 min. The homogenate was centrifuged for 60 min at 105500 × *g*, and the enzyme activity, protein content, and hemoglobin concentration of the supernatant measured.

Turtle blood was collected in heparinized Ringer's solution, washed 3 times, and the erythrocytes lysed by distilled water or by sonication. The hemolysate was centrifuged at 48000 × *g* for 20 min, and the hemoglobin, protein and carbonic anhydrase content determined.

Carbonic anhydrase activity was measured by a modification[7] of the method of PHILPOT AND PHILPOT[8]. Hemoglobin concentration was measured at 540 nm in a Beckman DU monochromator fitted with a Gilford absorbance indicator. Hemoglobin in the erythrocyte lysate was measured as the cyanmethemoglobin derivative using a factor of 1.44 (ref. 9). The oxyhemoglobin concentration in the mucosal cell supernatant was determined using a factor of 1.14 (ref. 9). Protein concentrations were determined by the Biuret method[10].

The results of the cell extractions and enzyme assays of four separate experiments are given in Table I. The carbonic anhydrase activity in each mucosal cell extract was measured in five aliquots ranging in volume from 50 to 200 μl. Enzyme activity was linearly related to aliquot size over this range. The value given, 7.22 enzyme units/ml, represents the mean of four mucosal cell preparations and is equivalent to 24.35 enzyme units/g wet weight of mucosal cells. The carbonic anhydrase activity in the erythrocyte lysate amounted to 398 enzyme units/g of cells (wet weight).

By measuring the concentration of hemoglobin in the lysate, the carbonic anhydrase activity was determined as 2.67 enzyme units/mg of hemoglobin. We also estimated by absorption spectroscopy the maximum concentration of hemoglobin in the mucosal cell extract as 0.054 mg/ml. This value does not consider the absorbance of 540 nm of compounds other than hemoglobin and is therefore spuriously high. It does allow one to estimate, however, that the maximum amount of carbonic anhydrase that could be contributed to the mucosal cell extract by the lysis of any contaminating turtle erythrocytes is only 0.14 enzyme unit/ml, or approx. 2 % of the total enzyme activity measured in the mucosal cell extract. Unless carbonic anhydrase, but not hemoglobin, diffused out of intact erythrocytes, the data on activity and hemoglobin contamination of the mucosal cell preparation suggest that the carbonic anhydrase detected must have come from some mucosal cell component rather than from the erythrocytes.

The molar concentrations of enzyme in the preparations from the mucosal cells and the erythrocytes were determined by titration with 2-*o*-chlorphenylthiadiazole-5-sulfonamide (CL 13580) by the method of KERNOHAN[11]. An aliquot size of each extract was chosen that gave approx. 1 enzyme unit activity. Aliquots were then assayed for activity following the addition of increasing amounts of 2-*o*-chlorphenyl-

TABLE I

CARBONIC ANHYDRASE IN EXTRACTS OF TURTLE ERYTHROCYTES AND BLADDER MUCOSAL CELLS

The values given are the means ± S.E. of four experiments. The assay mixture initially contained 13 μmoles of $NaHCO_3$ and 60 μg of phenol red gassed with 100% CO_2. The reaction rate was determined by the time required for the mixture to return to neutrality following the addition of 300 μmoles of Na_2CO_3 and 206 μmoles of $NaHCO_3$ (pH 9.0); total volume was 6 ml. Enzyme units were calculated by[7]: enzyme unit = (time, uncatalyzed) − (time, catalyzed)/(time, catalyzed).

Tissue	*Hb (mg/ml)*	*Enzyme units/ml*	*Enzyme units/g cell*	*Enzyme units/mg protein*	*Enzyme units/mg Hb*
Bladder	0.054 ± 0.01	7.22 ± 1.3	24.35 ± 1.7	1.03 ± 0.16	145.7 ± 10.0
Erythrocytes	5.95 ± 0.40	15.69 ± 2.5	398.0 ± 85	0.90 ± 0.19	2.67 ± 0.43

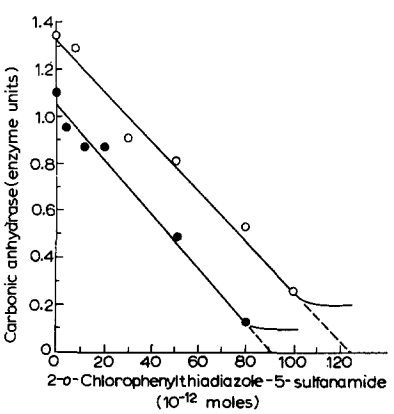

Fig. 1. Titration with sulfonamide of carbonic anhydrase activity in tissue extracts. These two representative experiments depict the titration of 50-μl aliquots of hemolysate (○) and of 100-μl aliquots of mucosal cell supernatant (●) with 2-*o*-chlorphenylthiadiazole-5-sulfonamide.

thiadiazole-5-sulfonamide. The addition of this sulfonamide, one of the most potent inhibitors of carbonic anhydrase[5], caused a linear decrease in enzyme activity in the range 100–12 % for the mucosal cells and 100–25 % for the hemolysate (Fig. 1). Extrapolation of the plotted lines to the abscissa indicates the concentration of carbonic anhydrase that was present in the assayed extract: 2.4 mM for the hemolysate and 0.9 mM for the mucosal cell extract. From these data we obtained values for the molar concentrations of carbonic anhydrase in the tissues. The mucosal cells contained $2.32 \cdot 10^{-9}$ moles/g wet weight and the erythrocytes, $22.8 \cdot 10^{-9}$ moles/g erythrocyte.

Although the characterization of enzymes in crude homogenates may be unreliable due to the presence of modifiers or inhibitors, these experiments demonstrate that there is an appreciable amount of carbonic anhydrase in the mucosal cells of the turtle urinary bladder. Previous attempts to demonstrate the enzyme in this tissue may have been unsuccessful because of (1) excessive dilution of the tissue in the course of the extraction and (2) the lack of any means for estimating whether small amounts of enzyme activity might be due to contamination from erythrocytes.

KITAHARA AND HOGBEN[6], working with cornea, and GONZALEZ AND SCHILB[12], with turtle bladder, have postulated that the sulfonamide-induced inhibition of Cl^- and/or HCO_3^- transport (in a system apparently devoid of carbonic anhydrase activity) could be due to an anion receptor having some structural similarity to the carbonic anhydrase molecule. The present demonstration of the enzyme activity in the soluble fraction of turtle bladder cells raises anew the possibility that carbonic anhydrase is related to the anion transport mechanisms (for Cl^- and HCO_3^-) in epithelial cells.

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