ABSTRACT  The effect of calcitonin (CT) and parathyroid hormone (PTH) on carbonic anhydrase (carbonate hydrolase, EC 4.2.1.1.) activity was tested in human erythrocyte hemolysates and with purified carbonic anhydrases I and II. The most important effect was on carbonic anhydrase II: CT showed a 2-fold increase and PTH showed a 50% decrease of carbonic anhydrase activity. This effect was observed at low hormonal concentrations and suggests the importance of CT in regulating carbonic anhydrase activity in the two important sites of CO₂ exchange, erythrocyte and lung.

Calcitonin (CT), a 32-amino-acid peptide hormone produced by C cells in the mammalian thyroid, is believed to interact with target cells predominantly in bone and kidney to modulate calcium and phosphate homeostasis. It inhibits bone resorption (destruction) both in vivo (1) and in vitro (2). Osteoclast activity is reduced, and in longer periods their number is reduced (3). CT acts in the kidney by enhancing the secretion of potassium, sodium, phosphate, calcium, and magnesium (4).

Specific binding sites have been demonstrated in rat bone and kidney membranes (5). The biological effect of this hormone has been shown to be mediated by adenylate cyclase in the plasma membrane of target cells. CT causes an increase in the concentration of cAMP in renal tubules (6) and in fetal calvaria (7) incubated in vitro.

Recently we have found that lung in mammalian vertebrates and the gill in fishes possess specific receptors for CT (8–10). This prompted us to investigate the function of CT in lung. Carbonic anhydrase (carbonate hydro-lyase, EC 4.2.1.1) mediates many important respiratory systems. It has been extensively studied in human erythrocytes, a most abundant source of this enzyme (11). Hence, we have examined the action of CT and parathyroid hormone (PTH) on the carbonic anhydrase of human erythrocytes. We report here the marked stimulation by CT and inhibition by PTH of carbonic anhydrase II.

The results suggest a function for CT in regulating gas exchanges in erythrocyte and lung.

MATERIALS AND METHODS

Hormones and Chemicals. Synthetic salmon CT (sCT; lot 54 F 0 335), human CT (hCT; lot 123 F 01021), and hPTH-(1–34) (lot 103 F 03241) were purchased from Sigma. CM Bio-Gel A, ion exchange resins, and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC coupling agent) were from Bio-Rad. p-Aminobenzenesulfonamid was from Serva, Heidelberg. All other chemicals were analytical reagent grade.

Enzymes. Freshly outdated erythrocytes provided by the local hospital were used to study carbonic anhydrase activity in hemolysates and to purify its isoenzymes. Hemolysates were prepared by repeated freezing and thawing of erythrocytes suspended in a buffer solution (40 mM Tris-HCl/2 mM ATP/3 mM MgSO₄, pH 7.4) (12). Isoenzymes B and C were separated by affinity chromatography as described (13, 14). Enzyme fractions were concentrated by vacuum dialysis and stored at 4°C. Enzyme concentrations were estimated from their absorbance at 280 nm using known extinction coefficients (11). Protein concentrations were determined by the method of Lowry et al. (15).

Enzyme Assay. Carbonic anhydrase activity was measured as described by Wilbur and Anderson (16) colorimetric assay for CO₂ hydration. This procedure involves injection of CO₂ to a buffered enzyme solution (pH 8.2) and measurement of the time required for the pH to drop by a certain amount (to pH 6.3, assayed by the color change of an indicator).

The activity is then proportional to (t_b/t_c), where t_b is the uncatalyzed reaction time and t_c is the time in the presence of a given aliquot of enzyme.

RESULTS

Time-Course of the CT-Stimulated Carbonic Anhydrase Activity. The effect of CT (0.1 µg/ml) on carbonic anhydrase activity in erythrocytes and on purified carbonic anhydrases I and II was measured at 20°C as a function of time (Fig. 1). Addition of CT slightly increased the carbonic anhydrase activity of hemolysates with a maximal and significant (P < 0.001) effect after 5 min of incubation. Carbonic anhydrase activity of such hemolysates is a result of activity of two isoenzymes (carbonic anhydrases I and II). CT stimulated one of these, carbonic anhydrase II, 2-fold (P < 0.001). The other, carbonic anhydrase I, was slightly inhibited (P < 0.001). The maximal effects were observed after 1 and 2 min of incubation at 20°C, respectively. Kinetic studies had shown that carbonic anhydrase II accounts for only 10% of the total enzyme (17). The slight inhibition of carbonic anhydrase I by CT can explain the low effect observed with hemolysates.

Dose-Response of hCA II to Calcium-Regulating Hormones. Fig. 2 describes the effect of hCT and sCT on carbonic anhydrase II activity.

After 2 min of incubation at 20°C, a concentration of sCT as low as 5 ng/ml stimulated the carbonic anhydrase activity. The maximal effect was observed for CT concentrations between 5 and 25 ng/ml and was a 2-fold activation compared to the basal value (enzyme activity in the absence of CT). Then, the carbonic anhydrase activity returned to the basal
level. For a concentration of 75 ng/ml, the activation was reduced to 20% of the basal value.

When tested under the same conditions, hCT activated the enzyme in the same manner as sCT. Although the two hormones differ by a factor of 50 in their hypocalcemic activity, there was no observed difference in their effects on carbonic anhydrase activity. Contrary to the dramatic stimulation observed with CT, hPTH inhibited carbonic anhydrase activity (Fig. 3). This inhibition was significant at hormonal concentrations as low as 5 ng/ml. The maximal inhibition, 50%, was observed at concentrations of PTH between 60 and 100 ng/ml.

**DISCUSSION**

The human erythrocyte contains two major forms of the enzyme, the high-activity form (with respect to CO₂), carbonic anhydrase II, and the low-activity form, carbonic anhydrase I (11).

Carbonic anhydrase catalyzes the reversible hydration/dehydration reaction of CO₂ and water. In the red cell, the enzyme serves primarily in CO₂ transport and excretion. CO₂ from respiring tissues enters the plasma and diffuses into the red cells, where it is rapidly hydrated to H⁺ and HCO₃⁻. In the lung, the reverse series of reactions takes place, and CO₂ is excreted. Erythrocyte carbonic anhydrase thus allows a large pool of otherwise slowly reacting plasma HCO₃⁻ to be utilized in CO₂ excretion.

In this study, we have demonstrated specific effects of CT and PTH on carbonic anhydrase II. CT activates and PTH inhibits the high-activity form of the human erythrocyte enzyme. In addition, the competition curve, Fig. 4, indicates that PTH interacts with the same site on the carbonic anhydrase molecule as does the substrate. These studies may be correlated with (i) a recent report showing that in rat liver carbonic anhydrase II is under testosterone and estrogen control (18) and (ii) a much earlier report (19) indicating an inhibitory effect of PTH upon calcium carbonate precipitation rates.

Regulation of carbonic anhydrase by calcium-regulating hormones is suggested by a number of reports that have shown that acetazolamide and CT have the same inhibitory effect on bone resorption (20). In addition, acetazolamide
inhibits PTH in vivo and in vitro (21, 22) and vitamin D-induced bone resorption (23). However, no direct effect of PTH or CT on osteoclasts in culture could be demonstrated (24). In kidney, there is a current controversy regarding the effect of PTH on bicarbonate excretion. Carbonic anhydrase is either inhibited (25) or unaffected (26) by PTH. Furthermore, the inhibition of bicarbonate and phosphate resorption does not seem to be mediated through carbonic anhydrase inhibition (27).

Recent studies of three siblings affected with the autosomal recessive syndrome of osteopetrosis with renal tubular acidosis and cerebral calcification have been reported (28). In an effort to explain the effects of the mutation producing this disorder, the authors observed the virtual absence of the high-activity enzyme in erythrocytes of patients. The level of carbonic anhydrase I was not reduced or affected. These observations link carbonic anhydrase II to osteoclast function and bone resorption.

The in vitro observation of an inhibitory effect of PTH on carbonic anhydrase II supports these conclusions and suggests that in lung, which is the site of the dehydration reaction, CT can affect the carbonic anhydrase activity. In this tissue, specific receptors for this hormone have been demonstrated (9), but the physiological action of CT was unknown. The results presented here suggest that CT, after binding to specific receptors in lung, could affect carbonic anhydrase activity. Carbonic anhydrase is associated in lung with pulmonary endothelial cell membranes located extracellularly facing the capillary lumen (29–32). The apparent function of the enzyme in this location is to promote the dehydration of serum bicarbonate to yield CO$_2$ that can readily diffuse across the endothelial barrier in expiration. Recently, Whitney and Briggle (33) suggested that membrane-associated carbonic anhydrase in lung is the same as that reported in the brush-border of the kidney tubules and is very similar to the high-activity enzyme (34).

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