Annu. Rev. Physiol. 1996. 58:523–38

MULTIPLE ROLES OF CARBONIC ANHYDRASE IN CELLULAR TRANSPORT AND METABOLISM

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KEY WORDS: carbon dioxide excretion, ammonia excretion, ureagenesis, gluconeogenesis

ABSTRACT

Carbonic anhydrase (CA) is a central enzyme to both transport and metabolic processes at the cellular level. In metabolically active tissue such as muscle, CA in the cytoplasm and on the sarcolemma appears to be important in facilitating CO_2 transport out of the cell. Membrane-associated CA, with an extracellular orientation, also appears to be important in acidifying the outer boundary layer through the catalyzed hydration of excreted CO_2. This facilitates cellular ammonia transport by providing H^+ ions for the protonation of NH_3, thus maintaining the *trans*-membrane NH_3 gradient. Mitochondrial CA is known to supply HCO_3^- for the initial reactions of gluconeogenesis and ureagenesis in mammalian tissues, but systematic comparative studies of CA as a metabolic enzyme are lacking. CA probably evolved as an enzyme of *trans*-membrane facilitated CO_2 transport and took on a secondary metabolic role later in metazoan evolution.

INTRODUCTION

Carbonic anhydrase (CA) is a primitive and ubiquitous enzyme found in virtually every tissue and cell type, in many subcellular organelles, and in organisms ranging from unicellular cyanobacteria through mammals (3, 23, 48, 50, 56, 57, 70, 93, 98). The enzyme catalyzes the reversible hydration/dehydration of CO_2 and water:

$$CO_2 + H_2O \overset{CA}{\rightleftharpoons} H^+ + HCO_3^- .$$

Because the reactants and products include both gaseous and ionic chemical species, CA could potentially be important for any physiological or biochemi-

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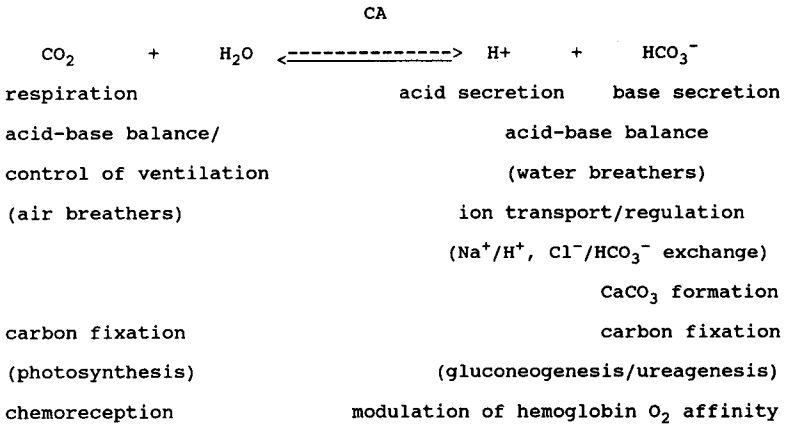

Figure 1 Suggested multiple physiological and biochemical roles of carbonic anhydrase. The physiological and biochemical processes in which CA may play a role are listed under the chemical species believed to be the net product of the catalyzed reaction. (Modified and expanded from Reference 50.)

cal process in which these species are used. Indeed, a large body of evidence supports the role of CA in a wide variety of processes from respiration to intermediary metabolism (see Figure 1 for a summary).

The two most heavily investigated areas of CA function, respiration (CO_2 transport and excretion) and ion transport, have been studied primarily on the systemic level. In the former the roles of erythrocyte and vascular CA have been well documented in the transport of CO_2 in the extracellular fluid (ECF) and its excretion across the respiratory epithelium (7, 30, 80, 83, 84). Ion transport has been most commonly studied in relation to the role of CA in systemic mechanisms of extracellular fluid ion regulation (48, 50, 61).

The limiting step in CO_2 transport, however, is known to occur at the cellular level. Although CO_2 gas is freely permeable across biological membranes, the more abundant chemical species in the ECF under physiological conditions, HCO_3^-, is not (25, 81, 92). The large ECF bicarbonate pool must first be mobilized to CO_2 gas before it can be excreted by diffusion. The uncatalyzed dehydration of HCO_3^- is very slow (t 1/2 = 25 s at 37°C) (24), and thus the limiting step becomes the conversion of HCO_3^- to CO_2. This was shown experimentally by Gutknecht et al (44): In the absence of CA, the limiting step in CO_2 transport across a membrane was the uncatalyzed dehydration of HCO_3^- to CO_2 in the boundary layer on the upstream side of the membrane. With CA present, HCO_3^- and CO_2 were maintained in instantaneous equilibrium, sustaining a high PCO_2 in the boundary layer, thus allowing the process to proceed at the rate of CO_2 diffusion through the membrane.

Therefore, the underlying principles that govern CO_2 transport are (*a*) the permeability differences between CO_2 and HCO_3^- and (*b*) the subsequent need for the rapid conversion of HCO_3^- to CO_2 gas. Two conclusions can be drawn from this information that bear directly on the interpretation of CA function. First, the rate-limiting step in CO_2 transport occurs at the site of the membrane, regardless of the complexity of the organism (e.g. unicells or metazoans). Second, CA localized in the boundary layer of the membrane is necessary for facilitated CO_2 diffusion. From this information it is apparent that the initial step in systemic CO_2 transport is the diffusion of CO_2 from the intracellular compartment of metabolically active tissue, across the plasma membrane, into the circulatory system. Furthermore, a combination of cytoplasmic and membrane-associated CA can be localized in such a way as to facilitate CO_2 transport across that membrane.

Although membrane-associated CA is believed to confer directionality on CO_2 transport from one biological compartment to another (e.g. see 70), cytoplasmic CA is believed to function simply in maintaining an instantaneous equilibrium between the chemical species of CO_2. Intracellular (including intraorganelle) CA has received attention recently for its potential role in carbon fixation for synthetic pathways of intermediary metabolism [e.g. gluconeogenesis and ureagenesis (16, 98); and photosynthesis in plants and symbiotic associations (2)].

What emerges from this initial discussion is an interesting concept that has not been given much consideration. The functions of CA in facilitated CO_2 transport across membranes and in supplying carbon for intermediary metabolism were already established when primitive unicellular and metazoan organisms were conducting gas exchange and ion transport across an undifferentiated integument. There was selective pressure for the evolution of CA function long before specialized respiratory and ion transporting epithelia evolved. Therefore, the primitive ancestral form of CA probably evolved to meet the CO_2 transport and metabolic demands on the cellular level. This review focuses on the multiple roles of CA in cellular transport and metabolism and on the influences those processes might have had on the evolution of CA function.

CA AND CELLULAR CO_2 TRANSPORT IN MUSCLE

Subcellular Localization and Isozyme Distribution

It was originally postulated that muscle tissue would not contain CA because it was believed that the rapid hydration of CO_2 to HCO_3^- would retard CO_2 transport into the blood (84). Perhaps for that reason, CA was not found in muscle until relatively recently (58, 108). Now it has been well established

that CA is present in both skeletal and cardiac muscle (for recent reviews, see 5, 10, 17, 33, 38, 42, 89, 90). In mammalian skeletal muscle, the CA II and III (and possibly I) isozymes are present in the cytoplasm, with the distribution correlated to the specific metabolic classification of the muscle fiber. CA III is found predominantly in type I muscle fibers (slow twitch, red, oxidative), and CA II is present in type II fibers (fast-contracting, white, glycolytic or mixed glycolytic-oxidative). In addition, there is a CA that appears to be similar to the mammalian red blood cell Type II isozyme associated with the sarcolemma of all muscle fiber types. Furthermore, there is also CA associated with the sarcoplasmic reticulum of all muscle fiber types. In contrast, mammalian heart muscle appears to have CA associated with the sarcolemma and intracellular organelles only (6).

Although mammalian systems have been extensively studied during the last fifteen years, there has been only one report for lower vertebrates indicating that frog white muscle has a CA type II isozyme (87). Other comparative studies are lacking, but preliminary information (85, 86; R Henry, unpublished data) indicates that other lower vertebrate (i.e. fish) and invertebrate (crustaceans) muscles contain both cytoplasmic and microsomal CA.

CA and Facilitated CO_2 Transport

Inhibition of CA in mammalian muscle is known to have a number of physiological consequences. Force of contraction is decreased for both twitch and tetany, and both contraction and relaxation times are increased. Inorganic phosphates are increased and intracellular pH (pHi) is decreased. From these and other results, muscle CA has been implicated as functioning in contraction, Ca^{2+}/H^+ transport by the sarcoplasmic reticulum (and therefore relaxation), neuromuscular transmission, metabolism and energetics, and facilitated CO_2 transport (34–37, 87). The latter role appears to have the most experimental support. Geers & Gros (37), using equations derived by Thews (94), calculated that there should be a reduction in pHi of about 0.13 units for cylindrically shaped muscle cells under conditions of CA inhibition. Their measured pHi depression of about 0.1 pH unit, after chlorzolamide treatment in isolated muscle fibers, was in close agreement. What is more interesting, however, is the fact that pHi was depressed in muscle fibers regardless of which CA isozyme was present; this strongly suggests that cellular CO_2 transport is a universal function of muscle CA, regardless of isozyme distribution.

Definitive evidence for the role of CA in cellular CO_2 transport outside of mammalian muscle is currently lacking, but circumstantial evidence appears to be supportive. Fish muscle is impermeable to HCO_3^-, and therefore CO_2 must diffuse across the sarcolemma in the gaseous molecular form (92). Furthermore, treatment of trout white muscle with the CA inhibitor acetazolamide

appears to cause CO_2 retention in the intracellular fluid (Y Wang, R Henry & CM Wood, unpublished data).

Although the function of muscle CA in facilitated CO_2 transport is generally accepted, the specific role of cytoplasmic or sarcolemma CA and the quantitative contribution of each to the overall process have not been established. In mammalian muscle, CA on the extracellular surface of the sacrolemma is believed to facilitate CO_2 transport from the interstitial space, across the capillary membrane, into the blood (39, 101). A recently isolated, perfused trout tail muscle preparation (Y Wang & CM Wood, unpublished data) has been used to study CO_2 excretion in conjunction with CA function. Selective inhibition of extracellular sarcolemma CA by the membrane-impermeant CA inhibitor quaternary ammonium sulfanilamide (QAS; 49) resulted in reduced CO_2 excretion from muscle fibers, indicating that both cytoplasmic and membrane-associated CA pools may be important in cellular CO_2 transport (Y Wang, R Henry & CM Wood, unpublished data).

CA AND CELLULAR NH_3 TRANSPORT

As with CO_2, ammonia transport and excretion has been most extensively studied on the systemic level. Because ammonia is the most abundant end product of nitrogen metabolism in aquatic animals, investigations into its excretion have focused on potential mechanisms of transport across the major epithelial surfaces (e.g. fish and invertebrate gills). The gills are also the organs of salt transport, thus early work centered on the relationship between ammonia and sodium transport. That work established the paradigm of Na^+/NH_4^+ exchange and implied that most of the ammonia excretion occurred in the ionized form by some mechanism of coupled transport (26, 68, 69). Two more recent advances, however, have changed the view of ammonia excretion: (*a*) Experimental evidence now supports the hypothesis that Na^+ transport at the gill occurs primarily by Na^+/H^+ exchange (27, 60, 62, 63), and more importantly, (*b*) the bulk of ammonia excretion appears to take place via NH_3 diffusion across the general epithelial membrane (1, 8, 9, 13, 28, 29, 64, 107).

In light of that information, our understanding of the behavior and transport of ammonia in solution becomes analogous to that of CO_2 (9). Ammonia exists both in gaseous (NH_3) and ionic (NH_4^+) forms. With a pK between 9 and 10, at physiological pH, about 97% of the total ammonia is in the protonated form in blood and intracellular fluid. Ammonia gas has both high water solubility and high permeability (values for NH_3 are between 10- and 100-fold higher than for NH_4^+) (9). The most abundant form does not appear to diffuse very readily, and the mobile (diffusable) form is present in very low amounts. The limiting step in ammonia transport occurs at the membrane, thus NH_3 diffusion

down a small but significant PNH_3 gradient (40–50 μtorr) (8, 9) will depend on mobilization (dissociation) of NH_3 from the much larger NH_4^+ pool on the upstream side of the membrane. Furthermore, reprotonation of NH_3 to NH_4^+ on the downstream side of the membrane will facilitate NH_3 diffusion by maintaining a minimum downstream PNH_3, or in other words, maximizing the *trans*-membrane PNH_3 gradient.

Acidification of the external boundary layer of the gill has been shown to be important in ammonia excretion in fish (102, 105). Treatments that reduce either the production of protons or their availability to NH_3 in the external boundary layer reduce NH_3 diffusion by up to one third. Because CO_2 is excreted in its molecular form and can be hydrated to form protons, it has been suggested that CO_2 excretion and NH_3 excretion are linked and that the protons come directly from the catalyzed hydration of CO_2 by CA localized on the extracellular surface of the gill (82, 105). At this point it is still questionable whether extracellular CA on the apical surface of the gill is necessary for acidification and subsequent NH_3 trapping. Amiloride treatment alone reduces NH_3 excretion by 30% (105), indicating that a significant fraction of the protons come from apical Na^+/H^+ exchange. This reduction happens in spite of a concomitant 70% stimulation of CO_2 excretion caused by amiloride. These results cast doubt on the role of branchial CA in acidifying the extracelluar boundary layer water for NH_3 excretion. An increase in CO_2 excretion in the presence of external CA should provide adequate amounts of H^+ for NH_3 protonation and, therefore, excretion; yet NH_3 excretion is inhibited. Furthermore, the supply of protons from respiratory CO_2 can be short-circuited at a number of steps prior to reaching the apical surface. Acetazolamide treatment in the blood reduces CO_2 excretion at the initial step (mobilization of HCO_3^- from blood) and reduces NH_3 excretion, while not having any effect on the apical membrane. The definitive experiments showing the presence and putative function of CA on the extracellular apical surface of the gill have not been performed.

CA may be more important in NH_3 excretion across the sarcolemma of muscle. In fish, one of the major metabolic sources of ammonia production, white muscle, contains high intracellular concentrations of total ammonia (6,000 μM). The NH_4^+ concentration gradient appears to be held in equilibrium by the *trans*-membrane potential, and there is also a very high PNH_3 gradient (400 μtorr) across the sarcolemma (106). For fish muscle at rest (low endogenous ammonia production), the bulk of ammonia excretion is believed to occur via diffusion of NH_3. In this case, CA on the sarcolemma, with an extracellular orientation, would facilitate NH_3 diffusion by acidifying the interstitial fluid boundary layer through the catalyzed hydration of CO_2. Again, definitive evidence for this function of CA is lacking, but preliminary results using QAS on an isolated, perfused trout muscle preparation indicate that

inhibition of extracellular CA reduces ammonia excretion from muscle (Y Wang, R Henry & CM Wood, unpublished data).

CA AND CELLULAR METABOLISM

Carbon dioxide and ammonia, the end products of carbohydrate and nitrogen catabolism, respectively, are primarily waste products that are eliminated from the animal by various routes of excretion. These chemical species are also used as substrates in a variety of metabolic pathways. CA can potentially play a role in metabolism in the specific steps that involve carbon fixation. Most of the catabolic reactions that produce carbon dioxide liberate CO_2 gas, and conversely, many of the carbon fixing reactions utilize HCO_3^- (98). Intracellular (and intra-organelle) CA, by maintaining a virtually instantaneous chemical equilibrium between CO_2 and HCO_3^-, could allow for some of the metabolically produced CO_2 to be shunted into synthetic pathways. Indeed, CA has been shown to function in providing HCO_3^- for the initial steps in glucose synthesis, fatty acid synthesis, general amino acid synthesis, and urea synthesis (16, 19, 20, 45, 46, 52, 54). Therefore, although CA is traditionally considered to be a transport enzyme, it is also an integral part of many biosynthetic pathways. CA is one of the few enzymes occupying a central role in both transport and metabolism, and as such it serves a molecular link between these two general processes (Figure 2). Most of the support for CA as a metabolic enzyme comes from work on mammalian systems, but two metabolic process have received considerable attention from a comparative standpoint: gluconeogenesis and ureagenesis.

CA and Gluconeogenesis

In mammals, glucose is the major energy source of the brain and the exclusive substrate for erythrocytes. After periods of intense exercise, when glycogen reserves have been depleted, lactate recycling to glucose through the Cori cycle is important in maintaining a continuous supply of fuel to metabolically sensitive tissue (55). The mammalian liver is the primary site of gluconeogenesis, and one of the initial steps in the pathway, the carboxylation of pyruvate via pyruvate carboxylase (PC), occurs exclusively in the mitochondria (4).

Carbonic anhydrase (CA V) is known to be present in mammalian hepatocyte mitochondria (22, 95). Furthermore, when intact hepatocytes were incubated under conditions in which gluconeogenesis begins with HCO_3^- fixation via PC (i.e. high lactate/pyruvate concentrations), treatment with CA inhibitors decreased glucose synthesis (20). Experiments on ^{14}C-labeled $NaHCO_3$ incorporation by PC showed a 65% reduction after CA inhibition (20). In contrast, under conditions in which gluconeogenesis begins independently of HCO_3^-

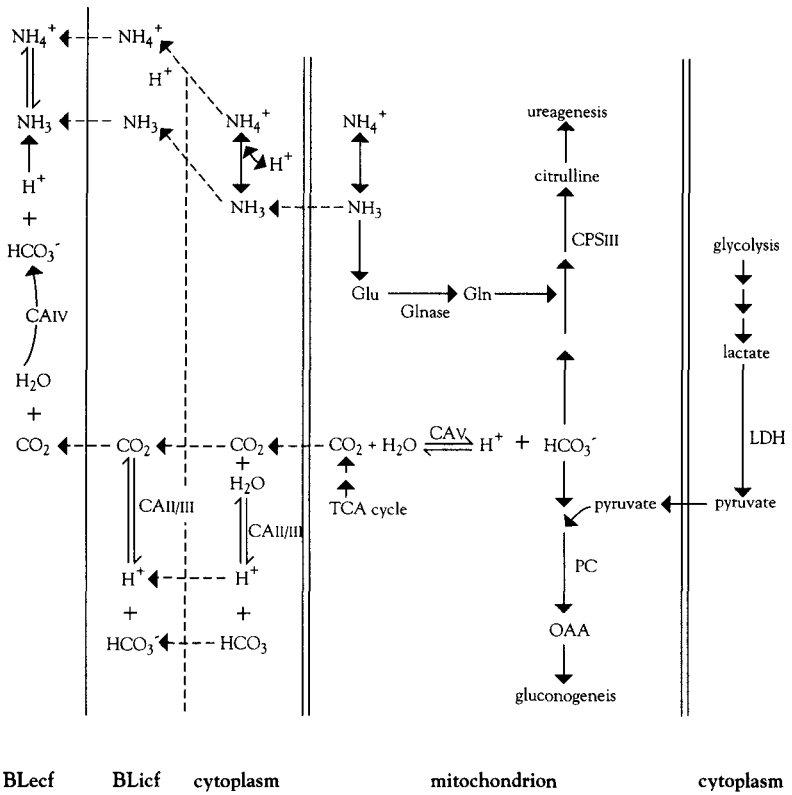

Figure 2 Composite schematic diagram indicating the multiple physiological roles for the different CA isozymes in CO_2 and NH_3 transport and in gluconeogenesis and ureagenesis. BL, boundary layer; ecf, extracellular fluid; icf, intracellular fluid. See text for a complete explanation.

(i.e. high glutamine concentrations), CA inhibition had no effect on glucose synthesis.

The role of mitochondrial CA V in gluconeogenesis is supported by studies on other mammalian tissues as well. Rat kidney is a highly gluconeogenic tissue that contains high levels of CA activity; guinea pig kidney, which lacks mitochondrial CA, has only 10% of the gluconeogenic capability (based on relative rates of glucose synthesis in starved animals) (18, 103).

The situation in lower vertebrates is less clear. First, the liver is not a primary site of gluconeogenesis. In lower vertebrates (reptiles and amphibians), skeletal muscle appears to be responsible for lactate recycling (14, 31, 32, 40, 41, 104). In frogs, surgical removal of the liver does not affect lactate elimination or

glycogen resynthesis (31). More specifically, in lizards, lactate produced by fast glycolytic fibers appears to be metabolized to glycogen preferentially in oxidative fiber types (41).

The liver of fish may also play a minor role in lactate-based gluconeogenesis. Elasmobranch (i.e. skate) hepatocytes do not utilize lactate to any significant degree in post-exercise gluconeogenesis (74), and teleost (i.e. toadfish) liver is responsible for clearing less than 2% of the post-exercise lactate load (96). At the very least, gluconeogenic capability appears to be spread among a variety of tissues, including liver, kidney, and muscle (73–76, 78, 97, 100).

There is very little known about the putative role of CA in gluconeogenesis in lower vertebrates. Even the most basic information on CA distribution among tissue types (e.g. muscle and liver) and its subcellular localization (e.g. mitochondrial or cytoplasmic) is lacking in all but one or two examples. Furthermore, even the metabolic pathways of glucose/glycogen resynthesis may not be consistant among species, as lower vertebrates have been shown to have very low levels of activity of two important gluconeogenic enzymes, pyruvate carboxylase (PC) and phosphoenol-pyruvate carboxykinase (PEPCK) (91, 98).

One study, in which the CA inhibitor acetazolamide was injected into intact chameleons and alligators, supports the role for CA in supplying HCO_3^- for pyruvate carboxylation (53). Gluconeogenesis in frog muscle has also been shown to be bicarbonate-sensitive but for different reasons. The difficulty in documenting significant levels of PC activity, and the results of ^{14}C tracer and inhibitor studies, led Connett (14) to suggest that lactate recycling occurs exclusively in the cytoplasm, possibly by carboxylation of pyruvate through the reversal of malic enzyme. Recent studies in fish white muscle suggest that the initial steps of gluconeogenesis proceed by reversal of another enzyme, pyruvate kinase (PK) (88), a pathway that was ruled out for frog muscle (14).

There has been only one study on CA and gluconeogenesis in invertebrates (51). No evidence was found linking CA to post-exercise lactate recycling in aquatic and terrestrial crustaceans. Although significant levels of CA activity were found in both muscle and hepatopancreas, key gluconeogenic enzymes [e.g. PC, PEPCK, fructose bisphosphatase, and even lactate dehydrogenase (LDH) in the hepatopancreas] were not detected in any significant amount (51, 65, 66). Lactate turnover rates were low, the percent of lactate resynthesized to glycogen was small (<20%), and ^{14}C from labeled lactate appeared in a variety of end products, suggesting multiple metabolic fates for lactate (51). Crustacean tissues seem to be poised more toward the metabolism of amino acids over carbohydrates (97), and this could partially explain why classical gluconeogenesis (via the Cori cycle) is so hard to detect. The use of acetazolamide in intact animals did not provide any evidence supporting a role for CA in gluconeogenesis (51).

Despite this conflicting and somewhat confusing information, some general conclusions about the evolution of gluconeogenesis and the role of CA can be drawn. First, it appears that the rate of gluconeogenesis is proportional to the overall metabolic rate of the animal (i.e. mammals → reptiles → fish → invertebrates) (15). Second, two of the three proposed pathways of gluconeogenesis involve carbon fixation, and the rate of CO_2 consumption is much higher than can be accounted for by that produced through mitochondrial respiration (16). It has been suggested that the CO_2 deficit is made up by the decarboxylation reactions in the gluconeogenic pathway itself [i.e. oxaloacetate (OAA) to PEP via PEP carboxykinase]. That reaction produces molecular CO_2, which could be utilized directly by malic enzyme reversal or utilized as HCO_3^- by PC after CA-catalyzed hydration. PK reversal, on the other hand, is independent of the CO_2/HCO_3^- reactions and therefore not involved in the action of CA.

Lower vertebrates and invertebrates appear to have the same capacity for glycolysis as mammals, but gluconeogenesis proceeds at much slower rates (15). This would imply equal selective pressure across broad phylogenetic lines for the release of quick energy during burst exercise but not for the recycling of metabolic end products. The difference may lie in the fact that the mammalian central nervous tissue is very sensitive to glucose; rapid gluconeogenesis (especially in the liver) could have evolved to meet the metabolic demands of a well-developed central nervous system. If so, then rapid conversion of CO_2 to HCO_3^- would have also been selected for, hence the importance of CA in ensuring that HCO_3^- availability does not become limiting in the initial step of pyruvate carboxylation. Outside of mammalian systems, however, the comparative importance of gluconeogenesis and the putative role of CA are subjects that remain open to investigation.

CA and Ureagenesis

The initial reaction of the urea cycle, the synthesis of carbamoyl phosphate from HCO_3^- and NH_3, takes place in the mitochondria. In mammals, the enzyme catalyzing this reaction, carbamoyl phosphate synthetase (CPS I), is localized to the mitochondrial matrix (67). The bicarbonate is supplied by a specific CA isozyme (CA V), which is colocalized in the mitochondrial matrix. CA was first found in guinea pig liver mitochondria (22), and its distribution now appears to extend to mitochondria of both liver and muscle across a variety of mammalian species (90, 95).

The use of CA inhibitors (e.g. acetazolamide) on isolated, perfused rat liver, hepatocyte, and mitochondria preparations has shown CA V to be physiologically important in supplying HCO_3^- for carbamoyl phosphate synthesis. Synthesis of citrulline in isolated mitochondria was reduced by 71% after treatment with acetazolamide (21), and urea synthesis in isolated hepatocytes was also

reduced by another CA inhibitor, ethoxyzolamide (19). The concentrations of the inhibitors needed to inhibit citrulline/urea were also close to those needed to fully inhibit mitochondrial CA activity, and an excess of inhibitor did not further suppress metabolite production. In isolated perfused liver, acetazolamide also decreased citrulline production by about 70%, but inhibition of both citrulline and urea synthesis was overcome by increasing the concentration of HCO_3^- to well above physiological levels (45–47). Furthermore, in intact rats in which CA was not inhibited, urea synthesis was dependent on NH_3 concentration only, unaffected by infusions of HCO_3^- (12). CA V and CPS I have been suggested to exist in the mitochondrial matrix as a complex (21), and carbamoyl phosphate appears to exert product inhibition on both enzymes (11). Taken together, these data provide strong support for a metabolic role for mitochondrial CA.

Since relatively few lower vertebrates (especially aquatic species) and fewer invertebrates are ureagenic, comparative studies on the role of CA in urea metabolism are scarce. Recently Mommsen & Walsh (77) found that a species of toadfish synthesizes and excretes urea during periods of environmental stress. A multifactorial experimental approach showed that urea synthesis is sensitive to HCO_3^- concentrations (99). Further study documented the presence of CA activity in both the cytoplasm and mitochondria of isolated toadfish hepatocytes, and treatment with acetazolamide resulted in a dose-dependent inhibition of urea synthesis (99). It was concluded that hepatocyte CA supplied HCO_3^- for the initial synthesis of carbamoyl phosphate, although the relative contributions of cytoplasmic or mitochondrial CA could not be determined. Remarkably, for all that is known about urea metabolism in elasmobranchs, little or no information on CA distribution and potential metabolic function is available. A similar situation exists for invertebrates: Some species are known to produce urea, but the potential role of CA has never been investigated.

THE EVOLUTION OF CA FUNCTION

Because CA was discovered in the mammalian red blood cell, it has been traditionally thought of as an enzyme of systemic CO_2 transport. As discussed above, however, multiple CA isozymes were in place and functioning at the cellular level long before the evolution of metazoans and integrated organ systems of transport. Recently, the argument has been made that CA can function as a true metabolic enzyme and that it may even have initially evolved to supply carbon for synthetic pathways (98). This is known to be the case in photosynthetic autotrophs (2), but for unicellular and more complexly organized heterotrophs (animals), it is not so simple. It is more probable that CA initially evolved as an enzyme whose function was in facilitated CO_2 transport on the cellular level. Evidence for this comes from both in vitro studies on

CO_2 diffusion in buffer solutions and across artificial membranes and from work on isolated muscle cells. In both cases, facilitated CO_2 diffusion occurs primarily at low PCO_2 (43, 59). In the presence of CA, and for PCO_2 values in the range of 5 torr, CO_2 diffusion is about five times higher than can be explained by the free diffusion of molecular CO_2. The rate increases exponentially as PCO_2 approaches zero, but it decreases toward the free diffusion rate of CO_2 as gas tension increases. The reason for this appears to be the dependence of facilitated CO_2 transport, not only on CA, but also on the concentration gradient of HCO_3^- and on an adequate amount of protons that are supplied by mobile buffers (43). As PCO_2 increases, the concentration gradients of HCO_3^- and mobile buffer decrease, and the transport of CO_2 is thus reduced, approaching the rate of free diffusion. It is possible that CA evolved as a result of selection pressure to maintain low intracellular PCO_2 in order to maximize facilitated CO_2 transport out of metabolically active tissue. Maintenance of an instantaneous equilibrium among CO_2 species via the catalytic action of CA at intracellular pH values of 7.0 and above (well above the pK′ value of 6.1 for the CO_2 system) would result in low PCO_2 and a constant supply of HCO_3^- for metabolic pathways.

If this was indeed the case in primitive organisms, then metabolism (and metabolic pathways of synthesis) would have evolved in a low PCO_2 environment. A number of key metabolic enzymes utilize HCO_3^- (42, 98), and as metabolic rates increased with the higher complexity of metazoan organization (and the invasion of the terrestrial habitat), it is also plausible that CA became more important in meeting the higher demand for metabolic carbon fixation. Regardless, the comparative and evolutionary systemic and cellular functions of CA represent areas that remain fertile grounds for investigation.

SUMMARY

The rate-limiting step in CO_2 transport for which CA is important occurs at the site of the membrane. Systemic CO_2 transport and excretion is comprised of a series of *trans*-membrane events strung together. CA appears to be just as important in facilitating CO_2 removal from metabolically active tissue into the ECF as it is in facilitating transport from the ECF across the respiratory epithelium. This is apparently accomplished by a combination of cytoplasmic (soluble) and membrane-associated CA. The function of CA in facilitated CO_2 transport from muscle also seems to play a related role in the removal of ammonia (NH_3) from tissues, and it may also function in facilitating systemic NH_3 excretion as well. Cytoplasmic and mitochondrial CA play an important role in supplying HCO_3^- for the initial steps in gluconeogenesis and ureagenesis in mammals. The function of CA as a metabolic enzyme has been documented

for ureagenesis in a select number of lower vertebrates but not invertebrates. The comparative importance of CA in gluconeogenesis is currently unclear.

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