Chapter 3
Targeting Carbonic Anhydrases in Cardiovascular and Pulmonary Disease

Erik R. Swenson, Akshay Kumar, Nimisha Kumar, and Bernardo V. Alvarez

Abstract Carbonic anhydrase (CA) is present within many cells of the heart, lungs, and the vasculature. An understanding of its roles in cardiac muscle and vascular smooth muscle, the vascular endothelium and lungs, was not fully appreciated until the 1980s and even presently more functions are being discovered. Despite its presence and roles in the physiological functioning of the cardiovascular and respiratory systems, its inhibition in some clinical circumstances may be therapeutic. In this chapter, we will review the expression and functions of the enzyme in the heart, lungs, and vasculature as background to a comprehensive discussion of how the enzyme may be targeted and its function altered in various disease afflicting these organs. While activators of CA exist, to date no studies of these compounds have been undertaken beyond a few neurological conditions. Targeting CA has largely involved the use of inhibitors, particularly acetazolamide, the first clinically approved inhibitor and still most widely used in its class. Interestingly, acetazolamide once thought to only bind to CAs at relevant clinical concentrations has many actions independent of CA with relevance to the heart, lungs, and vasculature. These include aquaporin blockade, activation of several membrane ion channels, and oxygen radical scavenging to name a few. Inhibition of carbonic anhydrase and actions of acetazolamide unrelated to enzyme inhibition have possible benefits in heart failure, myocardial infarction, acute lung injury, and systemic and pulmonary hypertension.

Keywords Carbonic anhydrase · Acetazolamide · Inhibitor · Aquaporin · Reactive oxygen species · Potassium channel · Acidosis · Diuretic · Vasodilation ·

E. R. Swenson (✉)
VA Puget Sound Healthcare System, Seattle, WA 98108, USA
e-mail: Erik.Swenson@va.gov

A. Kumar
Department of Cardiothoracic Surgery, University of Pittsburgh, Pittsburgh, PA, USA

N. Kumar
Department of Anesthesiology, University of Texas Health Science Center, Houston, TX, USA

B. V. Alvarez
Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada

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Pulmonary hypertension · Systemic hypertension · Hypoxia · Ischemia–reperfusion injury · Heart failure · Cardiac hypertrophy

3.1 Introduction

The discovery of carbonic anhydrase (CA) in red cells in the early 1930s and subsequent determination of its presence in many organs over the next several decades laid the foundation for the application of inhibitors of the enzyme to treat a variety of diseases with the introduction of acetazolamide in the 1950s. Other inhibitors arose out of synthetic efforts to develop even more powerful drugs than acetazolamide, some of which such as furosemide and hydrochlorothiazide interestingly were weaker inhibitors, but more effective diuretics acting on other tubular mechanisms of sodium reabsorption (Maren 1984). Importantly, it was the renal effect of acetazolamide on tubular CA that was quickly exploited in the treatment of edema in heart failure, as the first safe and non-toxic oral and intravenous diuretic agent. Despite the development of other equally or more potent CA inhibitors, acetazolamide remains still the most widely used drug in its class. Perhaps no other enzyme has been targeted in as many disease conditions as CA, owing to the myriad roles it plays in gas exchange, metabolism, membrane ion transport, acid-base regulation, and fluid balance. Given how inextricably linked acetazolamide and carbonic anhydrase are in the minds of most scientists and clinicians, it is ironic that acetazolamide, the prototypical and first clinically available inhibitor, appears also to have several targets and actions independent of CA inhibition that are relevant to cardiopulmonary physiology and disease, and likely in other organ systems as well. The intent of this review is to discuss the functions of CA in pulmonary and cardiovascular medicine, the positive and negative effects of enzyme inhibition in therapy, and in the case of acetazolamide, other actions of the drug that may be therapeutic alone or in combination with CA inhibition.

3.2 Carbonic Anhydrase Isozymes and Activity in the Cardiopulmonary System

Carbonic anhydrase is present in most cells of the vasculature, lungs, heart, and nervous system. The nervous system is included in this survey because of its critical role in regulation of breathing and the circulation. Depending upon the techniques, enzymic activity, histo- and immune-cytochemistry, and mRNA expression, all active isozymes are present. It took over four decades since its discovery for CA activity to be ultimately found in the vasculature, heart, and lungs; in part due to generally lower concentrations below the detection limit of early assay and histochemical methods and roles beyond classic gas exchange, acid-base regulation, and fluid and ion transport.

Heart: The first detection of CA activity in the heart was reported as far back as the 1940s by van Goor (1948), but the amounts were small and residual contamination with red cells could not be fully ruled out. These problems continued to confound efforts to definitively establish CA in the heart. Using an indicator dilution technique in cardiac perfusion studies, Zborowaska-Sluis et al. (1975) could not show an expanded CO_2 space in the myocardium consistent with the absence of CA, although they did so in skeletal muscle. Ellis and Thomas (1970) showed that acetazolamide reduced the rate of intracellular pH (pHi) change to an increase in CO_2 in the myocardium and conducting system. Moynihan (1977) after careful elimination of contaminating red cells was unable to report any activity. De Hemptinne et al. (1986) measured the transients in pHi and surface pH of Purkinje and myocardial fibers and found that acetazolamide altered the kinetics of surface pH changes. Using subcellular fractionation of cardiac myocytes after extensive saline perfusion of the heart to remove red cells, Bruns and Gros (1992) clearly established CA activity, and at roughly at the same time specific radiolabeled acetazolamide binding was demonstrated in the heart by positron emission tomography (Swenson et al. 1992, Swenson 1997).

In the heart, membrane-bound CA IV, CA IX, and CA XIV have been localized to different subcellular compartments of cardiomyocytes (Scheibe et al. 2006), and (CA IV) (Sender et al. 1998) to heart capillaries, and endothelium of coronary cardiac blood vessels. While CA XIV is predominantly localized in the longitudinal sarcoplasmic reticulum (SR), CA IX is mainly expressed in the terminal SR/t-tubular region (Orlowski et al. 2012; Scheibe et al. 2006), with CA IV localization detected in both SR regions. In the sarcolemmal membrane, only CA IV and CA XIV are present (Scheibe et al. 2006; Vargas and Alvarez 2012). In addition to CA IV, CA XIV, and CA IX, CA II has also been found (Brown et al. 2012; Vargas et al. 2013) and in diseased human hearts (Alvarez et al. 2013) hearts; and CA VB expression is reported in heart mitochondria (Fujikawa-Adachi et al. 1999; Vaananen et al. 1991; Vargas et al. 2016). CA III, the isoform with 1–5% of the catalytic activity of the other isozymes and more resistant to inhibition by most CA inhibitors, is also present in the heart (Coats et al., 2018), where it may play a role in anti-oxidant defense or in fatty acid metabolism.

Lung: Like the heart, discovery of CA in the lung was delayed by several decades until the first report in the 1950s by Berfenstam (1952). Relatively, little was further done, until the work of Lonnerholm (1982), Henry et al. (1986), and Nioka et al. (1988) using a variety of histochemical and biochemical techniques. Overall activity is low and only about 1% of that in red cells. CA IV is present in the vascular endothelium and located on the plasma facing aspect of these cells with its activity projected extracellularly (Carter et al. 1990; Fleming et al. 1994) and possibly into the parenchymal interstitial space. While initially not detected on alveolar epithelial cells (Effros et al., 1981a, b), Fleming et al. (1993, 1994) showed its presence in alveolar type II surfactant secreting cells. Chen et al. (2008) found evidence by both immunostaining antibodies and reverse transcriptase polymerase chain reaction (PCR) that CA II is present consistent with the earlier findings in whole lung tissue by Henry et al. (1986) and Lonnerholm (1982). CA II expression and activity is also

present in alveolar type I cells, which constitute most of the alveolar surface area (Chen et al. 2008). CA II, VI, IX, and XII are also present in mucus secreting serous and goblet cells and ciliated epithelium of the airways (Swenson 2000, Leinonen et al. 2004; Sugiura et al. 2009, Park et al. 2019). Lee et al. (2018, 2019) showed that CA IX in lung microvascular endothelial cells is critical to glycolytic metabolism that supports vascular growth particularly under acidotic conditions. Thornell et al. (2018) suggest that any airway epithelial CA activity is not available to the airway lining fluid by measurements in cell culture and in vivo of fluid pH. When CA is added to both models the change in airway pH is very rapid in response to a change in ambient CO_2, but slow and not affected by CA inhibitors under control conditions. Livermore et al. (2015) found CA expressed in airway neuroepithelial bodies, which are a discrete set of cells with both O_2 and CO_2 sensing properties that may contribute to the control of ventilation. The lung also has a rich variety of immune cells residing in the interstitium and alveolar space. CA IV is detectable in alveolar eosinophils (Wen et al. 2014), CA II in alveolar macrophages (Hudalla et al. 2019) and CA I, II, and III in neutrophils (Campbell et al. 1994).

Vasculature: The vasculature of the heart, lungs, brain, and specialized chemoreceptor sites constitutes a smooth muscle layer in vessels regulating vascular tone upstream (arteriolar) and downstream (venular) of the capillaries and a continuous endothelial cell layer from artery to vein. The first histochemical evidence of CA in systemic arterial smooth muscle was CA III (Jeffrey and Carter 1980). Numerous other non-vascular smooth muscle tissues (Berg et al. 2004a, b) have several forms of CA by immunocytochemical staining. In vascular smooth muscle of the bovine aorta stripped of its endothelium, CA activity is present at a very low level of 3.5 units/g tissue. By comparison other tissues with CA have activity in the range of 20–1600 unit/g. CA I is the most abundant isozyme, constituting about 80% of the activity followed by smaller amounts of CA II and only a small fraction attributable to CA III (Berg et al. 2004a, b). The endothelium of blood vessels, which elaborates numerous vasoconstricting and vasodilating substances, expresses several CA cytosolic and membrane-bound isozymes. These in the microcirculation include CA I, II, and III (Mahieu et al. 1995) and several membrane-bound CAs, principally CA IV, but also CA XII and CA XIV (Fleming et al. 1993; Agarwal et al. 2010). In lungs with pulmonary hypertension, local hypoxia is associated with CA IX expression in the medial layer of vascular cells (Howard et al. 2012). Little is known about CA expression in the endothelium of larger resistance vessels, those more importantly involved in blood flow regulation.

3.3 Effects of CA Inhibitors Independent of CA Inhibition

As alluded to above, CA inhibiting sulfonamides including acetazolamide may have effects independent of CA inhibition and considerable evidence is emerging that these actions, either with or without concomitant CA inhibition could be useful in other diseases associated with hypoxia, edema, and ischemia.

Aquaporin (AQP) inhibition. Virtually all cells have specific membrane water channels that contribute to intracellular osmoregulation and extracellular water regulation, and in many organs, they contribute to transepithelial fluid transport. Of the many members of the aquaporin family, AQP-1 and AQP-4 are of special interest with regard to acetazolamide. Both AQP-1 and AQP-4 are expressed in the brain (Benga and Huber 2012), particularly in the choroid plexus and astrocytes, respectively, where they are involved in CSF production (Ameli et al. 2012) and CBF regulation (Nakada 2015), and brain extracellular fluid and water homeostasis (Igarashi et al. 2013). Inhibition of AQP-4 and genetic deletion of AQP-4 are protective against some forms of cerebral edema, and upregulation of these aquaporins causes both cerebral and peripheral nerve edema (Igarashi et al. 2013). AQP-1 is the major isoform in red cells, the kidneys, and peripheral nerves and is intimately involved in whole body osmoregulation.

Acetazolamide and other CA inhibitors may alter aquaporin-mediated water conductance by three possible mechanisms: direct blockade of the water channel of AQP, inhibition of CA that co-localizes with AQP and aids in water formation, and downregulation of AQP gene transcription and translation. With regard to AQP-4 and to some extent AQP-1, acetazolamide in clinically relevant uM concentrations directly blocks water flux across the plasma membrane of oocytes or liposomes in vitro (Ameli et al. 2012; Huber et al. 2009; Tanimura et al. 2009), but not all studies confirm these findings in oocytes (Sogaard and Zeuthan 2008; Yamaguchi et al. 2012) or more complex cells (Yang et al. 2008). Somewhat surprisingly, Tanimura et al. (2009) found that methazolamide was inactive in blocking water flux, despite its very close structural similarity to acetazolamide; differing only by a methyl group substitution on the thiadiazole ring. Similarly, n-methyl acetazolamide, with a methyl substitution on the sulfonamide binding directly to the active site of CA, does not alter oocyte water fluxes mediated by AQP-1 and 4 (Huber and Swenson, unpublished data). Most recently, it has been shown that AQP-1 covalently binds to CA II and in frog oocytes doubles the rate of water flux. This potentiation requires CA activity because co-expression of a catalytically inactive CA mutant that still binds to AQP-1 does not increase water flux (Vilas et al. 2015). How a tight proximity and co-localization of CA II with AQP-1 enhances water conductance is not clear, but it may involve selective channeling of water molecules concentrated near CA to the H_2O channel of aquaporin.

Acetazolamide also inhibits AQP-1 and AQP-4 gene and protein expression in models of brain and cardiac injury (Ran et al. 2010), as well as possibly accelerating its proteasomal degradation via ubiquitination (Zhang et al. 2012). It has been reported to reduce vasogenic and cytotoxic forms of cerebral edema in animal models (Guo et al. 2012).

Interestingly, aquaporins may also serve as channels for small uncharged gas molecules, such as nitric oxide (NO), NH_3, O_2, and CO_2 (Herrera and Garvin 2011). It is not known if acetazolamide and other CA inhibitors block aquaporin-mediated CO_2 diffusion across the cell membrane, but if so, this may represent another mechanism by which acetazolamide would cause intracellular CO_2 retention and acidosis to stimulate ventilation as discussed earlier.

Radical oxygen species modulation: Hypoxic exposure equivalent to typical high altitudes increases ROS formation (Swenson 2016). Acetazolamide, a heterocyclic thiadiazole, might work as an antioxidant given that other numerous compounds containing a 1-3-4 thiadiazole ring are ROS scavengers (Prouillac et al. 2009). Natural defenses against ROS include a number of antioxidant proteins that are upregulated by the gene transcription factor, nuclear related factor-2 (Nrf-2) (Lisk et al. 2013). Recently it has been shown that methazolamide, but surprisingly not acetazolamide, at clinically relevant dosing activates Nrf-2 in the brain and decreases hypoxic-mediated cerebrovascular leakage in a rat model (Lisk et al. 2013). Whether this difference between the drugs just represents the greater lipophilicity of methazolamide over acetazolamide and great BBB penetrance, or some unique attribute of methazolamide will require more extensive pharmacological investigation. Several models of ROS-mediated cellular or organ injury have shown that acetazolamide and methazolamide reduce cerebral damage, apoptosis, neuronal dysfunction and inflammation (Shah et al. 2013; Wang et al. 2009).

Hypoxia-inducible factor (HIF): The master hypoxic transcription factor, HIF-1, is important in surviving hypoxic stress. When normoxic rats were given very large doses of acetazolamide (50–100 mg/kg), HIF-1 alpha was upregulated in brain tissue (Xu et al. 2009). Another study of a similar degree of acidosis (pH ~7.0) in cultured cells found moderate up-regulation of HIF-1 (Willam et al. 2006). Whether the very slightly lower blood pH with acetazolamide compared to those not treated (usually about 0.05 units) causes any differences in HIF-1 activity or its metabolism remains unknown. It will be important to establish whether acetazolamide with more typical administration under the acid–base conditions of high altitude and other diseases alters HIF expression since recent work suggests that the genes for AQP-1 and 4 are HIF responsive and have HIF binding sites (Abreu-Rodriguez et al. 2011).

HCO_3-sensitive soluble adenyl cyclase: Recently it was reported that acetazolamide at a concentration (500 uM) higher than usually attained with typical clinical administration (100 uM) increased cyclic AMP in ciliary epithelial cells of the eye. The mechanism of action appears to be a stimulation of bicarbonate-sensitive soluble adenyl cyclase activity (Rahmne et al. 2013), which may be also involved in metabolic communication between astrocytes and neurons (Choi et al. 2012), regulation of mitochondrial oxidative metabolism (De Ramso et al. 2015; Acin-Perez et al. 2009), cholangiocyte secretion (Strazzabosco et al. 2009), and mitochondrial calcium homeostasis (Tanzaarella et al. 2019).

Calcium-activated potassium channel (BKCa): Acetazolamide and some other, but not all, potent CA inhibitors at similarly high concentrations also activate a large capacitance calcium-activated potassium channel in human blood vessels to hyperpolarize vascular smooth muscle and cause vasodilation (Pickkers et al. 2001), protect against hyperkalemia (Tricarico et al. 2013), and treat such disorders as hypokalemic periodic paralysis and myotonic disorders (Tricarico et al. 2006).

3.4 CA Functions in the Normal Heart

3.4.1 CO_2 Elimination

A principle role of CA is to increase the rate of transfer of CO_2 from its production in the mitochondria and diffusion through the cytoplasm to the capillaries of the heart. While earlier studies put the total activity of heart CA at very low values, in more recent work, Arias-Hidalgo et al. (2017) have calculated that intracellular CA activity is enough to accelerate CO_2 hydration and HCO_3 decarboxylation rates by 5,000-fold, a value not surprising for an organ with a constant high metabolic rate even at rest. With exercise, CO_2 production and need for rapid extracellular disposal increases 5–10 times, a rate that is facilitated by a very high transmembrane CO_2 permeability of 0.10 cm s^{-1}. To put this newer work in perspective, the value for red cells is on the order of 20,000-fold. The efficient disposal of CO_2 in the heart is the result of both intracellular CA II and membrane-bound forms of CA IV, CA IX, and CA XIV acting to increase CO_2 elimination by 'facilitated diffusion of CO_2' (Arias-Hildalgo et al. 2017; Schroeder et al. 2013) within the cytosol by rapid formation of bicarbonate to co-diffuse with CO_2 in the cytosol and then cross the membrane as CO_2 from rapid generation from bicarbonate. This can be considered a microscopic analogy of the same role that red cell CA plays in increasing the efficiency of CO, movement from the tissues to the lungs, i.e., by permitting greater flux of CO_2 over smaller PCO_2 gradients through rapid formation and consumption of bicarbonate.

3.4.2 Cardiac pH Homeostasis

Another physiological function of CA in the heart appears to be maintenance of both extracellular (pHe) and intracellular pH (pHi) homeostasis. However, CA does not appear to make a significant contribution to the regulation of steady state pH_i under normal in vitro perfusion conditions (Vandenburg et al. 1996), but no in vivo data exist. Related to specific CA locations in the heart, an extracellularly active CA (most likely CA XIV and CA IX) increases the availability of the extracellular CO_2/HCO_3^- buffer system to affect the surface pH in unstirred layers by accelerating the reversible CO_2 hydration reaction (de Hemptinne et al. 1986). The CA inhibitor acetazolamide (ACTZ) slows the pH_i response after CO_2 change in mammalian myocardium and Purkinje fibers (Ellis and Thomas 1976; Lagadic-Gossman et al. 1992) as well as the subsequent pH_i recovery (Lagadic-Gossmann et al. 1992).

Intracellular CA facilitates and potentiates the activity of a number of pH regulatory membrane transporters such as Na^+/H^+ (NHE) and anion exchangers (Villafuerte et al. 2014), as well as effective H^+ mobility, regulating the spatiotemporal uniformity of pH_i (Spitzer et al. 2002; Vaughan-Jones et al. 2002, 2006, 2009). Without CA activity, intracellular HCO_3^--dependent buffering, membrane HCO_3^- transport, and the CO_2-HCO_3^- shuttle are severely hampered (Vaughan-Jones and

Spitzer 2002). There is a functional partnership between CA and HCO_3^- transport, and one physiological role for CA is to act as a pH-coupling protein, linking bulk pH to the allosteric H^+ control sites on sarcolemmal acid/base transporters (Vaughan-Jones and Spitzer 2002). More recently, physical and functional coupling between membrane-associated CA IV, CA IX, and CA XIV and the NBC Na^+/HCO_3^- cotransporters and Cl^-/HCO_3^- exchangers was demonstrated in isolated cardiomyocytes and ventricular muscle of mammalian hearts (Alvarez et al. 2007; Orlowski et al. 2012; Vargas and Alvarez 2012; Vargas et al. 2013; Morgan et al. 2004), suggesting a pivotal physiological role of CA in myocardial pH regulation. Also intracellular CA II in heart papillary muscles forms a complex with the NHE1 Na^+/H^+ exchanger and contributes to cardiac muscle contractile activity (Krishnan et al. 2015; Vargas et al. 2013).

3.4.3 Metabolism-Perfusion Matching

It has been recognized that there are marked spatial heterogeneities in regional blood flow and metabolism even in the healthy heart (Frazen et al. 1988; Gonzalez and Bassingthwaighte 1990) and that these vary with time (King and Bassingthwaighte 1989). The nature and origin of the fluctuations and spatial differences are not at all clear, but the implication is that there must be local control of regional perfusion in relation to metabolism to prevent metabolism-perfusion mismatch. Local accumulation of metabolites and supply of substrates such as oxygen have long been thought to provide some of the regulatory signals although the mechanisms are still not well understood and other mechanisms are probably operative (Dhainaut et al. 1991).

Carbon dioxide is a metabolite of both aerobic and anaerobic metabolism and contributes to the regulation of local blood flow-metabolism matching. Fiegl and colleagues (Katz and Fiegl 1987; Broten and Fiegl 1992; Broten et al. 1991) have thoroughly investigated the determinants of coronary blood flow in large regions of the heart and have shown that changes in coronary venous PCO_2, which are an accurate measure of capillary and tissue PCO_2 (Katz and Fiegl 1987), when combined with the accompanying PO_2 change, account for one quarter of the auto regulatory flow response to changes in coronary artery pressure (Broten and Fiegl 1992) and 40% of the blood flow response to induced pacing (Broten et al. 1991). Waxes et al. (1996) found similar results with epinephrine infusion and also demonstrated that these PCO_2 changes in myocardial blood glow are the consequence of the accompanying pH change (Wexels et al. 1986).

These findings suggest a possible rationale for CA activity in the heart as shown for ventilation-perfusion (V_A/Q) matching in the lungs (Swenson et al. 1995, 1993). Myocardial CA activity either in the capillary endothelium and/or the myocardial extracellular space would promote the more rapid attainment of a new pH when myocardial metabolism alters the prevailing PCO_2. The new pH, in turn, then would increase or decrease blood flow in appropriate direction to establish a better match between local perfusion and metabolism.

3.4.4 Fatty Acid/Lactate Uptake

Free fatty acids are the primary cardiac fuel, but the heart also utilizes lactate and pyruvate under normoxic conditions (Goresky et al. 1994; Poole et al. 1989). Lactate, pyruvate, and the free fatty acids are virtually ionized in vivo, since their dissociation constants are several pH until lower than the physiological pH range. In model lipid bilayer systems, the entrance of unionized free fatty acids is several offers of magnitude faster (seconds vs minutes) than that for the charged form (Kamp and Hamilton 1992). In addition to this simple passive mechanism for free unionized acid transfer, there are also specific transport proteins in the membranes of the cardiac capillary endothelium and myocyte that mediate a high extraction (40–50%) of free fatty acids from plasma in association with albumin (Chu and Montrose 1995) and of lactate and pyruvate (Poole et al. 1989). Whether these transporters preferentially utilize the unionized acids is unknown. In some organs, such as the colon (Chu and Montrose 1995; van Englehardt et al. 1993), the rate of uptake of free fatty acids appears to be a function of pH and dependent upon transport mechanisms secreting acid or absorbing base suggesting the quantitative importance of undissociated free fatty acid diffusion. To the extent that free fatty acid uptake (and lactate) in the heart is dependent upon unionized acid movement, there will be a perturbation of the internal and external pH as undissociated acids carry protons across into the cell, as has been shown in the colon (Chu and Montrose 1995; Titus and Ahearn 1992). The resulting intracellular acidification and external alkalization in the vicinity of the membrane will slow the further movement of free fatty acids and lactate. In the presence of other buffers, CA activity will to dissipate and blunt development of rate limiting pH gradients. At this time, it is unknown whether cardiac free fatty acid uptake is dependent upon CA and would be reduced by CA inhibition as has been shown in the colon (Hatch 1987).

3.4.5 Myocardial Contractility and Calcium Mobilization

Myocardial contractility and relaxation is dependent upon the rapid release and reuptake of calcium during the contractile cycle. The presence of CA IV in the terminal sarcoplasmic reticulum (SR) has a predicted membrane orientation with its catalytically active site facing the interior of the SR. Thus, by accelerating the CO_2/HCO_3^- interconversion inside the SR, CA IV can provide buffering of H^+ changes during Ca^{2+} release by H^+/Ca^{++} exchange during systolic contraction as well as fast delivery of H^+ for Ca^{2+} reuptake in diastolic relaxation (Scheibe et al. 2006). In isometrically-contracting perfused isolated papillary muscles, the two potent CA inhibitors, chlorzolamide (CLZ) or ethoxzolamide (ETZ), caused a reversible decrease in isometric force in muscles maintained in a Krebs–Henseleit bath solution at 20 °C (Geers and Gros 1995). Inhibition of CA with both the potent free membrane-diffusible CA inhibitor ETZ and potent membrane diffusion restricted CA inhibitor 11,366

(benzolamide, BZ), had no effect on baseline pH_i or contractile performance of isolated Langendorff-perfused hearts, under normal perfusion conditions (Vandenberg et al. 1996). Similarly, ETZ did not change the isometric force development of rat papillary muscle after 20 min of incubation in a CO_2/HCO_3^- buffered solution at a more physiological (30 °C) temperature (Vargas et al. 2013).

Given the above in vitro studies, many of which but not all showing deleterious effects of CA inhibitors on some aspect of myocardial function, it would be predicted that significant CA inhibition in the heart should reduce exercise capacity by limiting myocardial contractility and relaxation. The failure of cardiac output to rise appropriately that might arise due to CO_2 retention, intracellular acidosis and impairment of calcium turnover. Studies in exercising humans and horses, however, surprisingly show little to no reduction in the normal increase in cardiac output with heavy exercise. In humans exercising to maximum levels over 10–15 min 3 mg/kg of acetazolamide does not reduce exercise capacity or cardiac output either in normoxia or while breathing 12.5% oxygen (Jonk et al. 2007) despite the slight systemic metabolic acidosis generated by the drug and resultant increased ventilation. These results at relatively low dosing, were essentially no different in exercising race horses administered 30 mg/kg in showing no impairment in maximal oxygen uptake and cardiac output (Vengust et al. 2006, 2010, 2013). The only suggestion of an impairment of exercise capacity in horses was the finding that time to fatigue with running close to maximal oxygen uptake was shortened with CA inhibition (Rose et al. 1990). This may have been due to the greater acidosis in the drug treated horses and cardiac output was not measured in this study. Thus, it remains something of a mystery that CA inhibition has little impact on cardiac output in normal humans and animals even with the demands of heavy exercise. One explanation, albeit difficult to determine, is whether acetazolamide has enough penetrance into heart muscle to reach critical inhibiting concentrations. However, doses of acetazolamide >20 mg/kg alter function in many other organ systems in which carbonic anhydrase is present and involved. Two other CA inhibitors with greater potency and cellular penetrance, ethoxzolamide, and chlorzolamide have not been studied in vivo, but a few studies in vitro (see above) do demonstrate some decrease in myocardial contractility. No investigations have been performed in animals with genetic deletion of any isozyme or humans with CA II deficiency. In the case of CA II deficiency the numerous other complications of global deficiency (metabolic acidosis, osteopetrosis, cerebral calcifications, and growth retardation), there might be other reasons that these adults and children would not be able to exercise at high levels.

3.5 CA Functions in the Normal Lung

3.5.1 Alveolar Carbon Dioxide Elimination

Enns and Hill (1983) found that CO_2 diffusion across non-perfused blood-free lung tissue is decreased roughly 40% with CA inhibition, consistent with facilitated diffusion of CO_2 as already described above for the heart. Evidence supporting this role has come from studies of isolated lungs perfused with blood-free solutions alone or those to which sulfonamide-resistant plant CA had been added. Under these circumstances CO_2 excretion can be reduced by 20–75% by lung tissue CA inhibition (Klocke 1978; Hanson et al. 1981; Enns and Hill 1983; Crandall and O'Brasky 1978; Heming et aI. 1986, 1994; Schunemann and Klocke 1993). Based upon data with permeant and impermeant inhibitors, CA dependent CO, excretion in bloodless lungs depends upon CA IV on the cell membrane of endothelial cells facing the plasma (Heming et al. 1993, 1994). Early results of Heming et al. (1986) with dextran bound sulfonamides suggesting that intracellular CA activity was important has been reinterpreted by this group (Heming et al. 1994) since their extremely large dextran inhibitors did not have access to CA IV in caveolae, small invaginations of the plasma membrane that are still accessible to smaller cytosolic-impermeant compounds but may be beyond the reach of large molecular weight endogenous plasma CA inhibitors.

The situation in vivo greatly reduces the role of either intracellular or membrane bound lung CA in enhancing CO_2 elimination by facilitating CO_2 diffusion or catalyzing plasma HCO_3 dehydration. Firstly, there is no evidence that CO exchange in the lung is predominantly diffusion limited given the thinness of the normal alveolar capillary barrier (Effros et al. 1981a, b). Plewes et al. (1976) found no effect of CA inhibition on transpleural CO_2 excretion in the isolated blood-free perfused lung. Since the gases had to pass the alveolar septa, any effect of facilitated diffusion should have been observed. Secondly, many of the above mentioned lung perfusion studies employed conditions (high PCO_2 gradients, reduced perfusate buffering, no erythrocytes, and no concurrent oxygen exchange) that artificially enhance any contribution of facilitated CO_2 diffusion and plasma HCO_3 dehydration to total CO_2 output (Heming et al. 1994). Thirdly, since red cells have 100 times the enzymic activity of lung tissue and ten times the buffering capacity of plasma it is not surprising that 90% or more of capillary CO_2 traverses the red cells. The confinement of CA within red cells gives it immediate proximity to hemoglobin, the only buffer for the CO_2-HCO_3 reactions of a sufficient amount (in part due to its oxylabile character) to sustain physiological CO_2 output. These conclusions are supported by model simulations of lung gas exchange by Crandall and Bidani (1981) and Mochizuki et al. (1987) which calculate that lung tissue CA can maximally account for no more than 5–10% of normal CO_2 elimination.

In a provocative study, Kawai et al. (2015) proposed that lung CO_2 excretion is dependent upon a blood-flow mediated activation of vascular F1/FO ATPase which generates a H^+ to combine with plasma bicarbonate to generate CO_2 for diffusion across the alveolar capillary barrier. In this scheme, red cell CA, the majority of

CA within the perfused lung is not necessary for CO_2 exchange. They base their theory on the results of a specific inhibitor of F1/FO ATPase, piceatannol which they show decreases CO_2 excretion in an isolated perfused lung. They find that acetazolamide also decreases CO_2 excretion, since it would be necessary for the generation of carbon dioxide from the reaction of H^+ and bicarbonate. The problem with this novel mechanism is that piceatannol, a phenolic compound, is likely a CA inhibitor (Swenson et al., unpublished results) as are other compounds with structural similarity to piceatannol (Innocenti et al. 2010) Thus the classical understanding of CO_2 excretion in the lung is not necessarily overturned by this study.

The few relevant in vivo experiments show only a minor small contribution of lung CA to CO_2 elimination. Swenson et al. (1993) found that benzolamide, a highly impermeant CA inhibitor but active against CA IV, reduced CO, output by a non-statistically significant 8% in the dog lung in a first pass injection compared to the large decrement with ethoxzolamide. Moreover, there was no further effect of a band 3 inhibitor which was added to force more plasma CO_2-HCO_3 interconversion by limiting red cell Cl-/HCO_3 exchange, as predicted by Bidani (1991). One mg/kg benzolamide did not increase the venous to arterial CO_2 difference in anesthetized dogs (Swenson et al. 1993) although Cardenas et al. (1998) found that 2 mg/kg reduced CO_2 output by about 10%. It is interesting in the context of gas exchange efficiency to note that hypercapnia, a stimulus expected to possibly increase CA IV, does not increase its expression in lung endothelial cells (Rounds et al. 1997) despite an increase in other membrane associated proteins.

Healthy men given a very low dose of acetazolamide (3 mg/kg iv) to avoid significant red cell CA inhibition, but sufficient to inhibit vascular CA IV, showed that only with maximal exercise was there any detectable decrease (5%) in CO_2 excretion (Korotzer et al. 1997). Whether this was due to decreases in oxygen consumption or true CO_2 retention is not clear because there was a non-statistically significant decline in maximal oxygen consumption ($p = 0.06$) and no significant differences in arterial CO_2 and ventilation. The only study to find a large contribution of lung CA to CO_2 elimination in the presence of red cells was that of Klocke (1997) in an isolated blood perfused lung. In a setting of no concurrent oxygen uptake and a non-physiological PCO_2 gradient of almost 40 mmHg, he found that lung CA inhibition alone reduced CO, output by 44%. Both of these non-physiological conditions may have served to enhance the contribution of lung CA. Further definitive experiments ideally should use absolutely impermeant CA inhibitors, such as F 3500 (Conroy et al. 1996) under steady-state in vivo conditions.

3.5.2 Lung Fluid Exchange and pH Regulation

The lung parenchyma and airways, especially during their development, actively secrete or reabsorb fluid (Strang 1991; Dorrington and Boyd 1995). The fetal lung secretes an acidic (pH ~6.27, $HCO_3 = 2.7$ mM) poorly buffered fluid (Adamson et al. 1969) thought necessary for optimal growth and surfactant function at the time

of birth and transition to air breathing (Strang 1991). The mechanism of acidic fluid secretion by the alveolar epithelium appears to be one involving active H^+ secretion via either Na^+/H^+ exchange or H^+/K^+ ATPase (Strang 1991). The capacity for acid secretion is large since fetal lungs of lamb can reduce intra-alveolar HCO_3 from 60 to 3 mM over 4–5 h (Olver and Strang 1974).

During lung growth in utero, CA II and IV begin to appear in mid-term and reach peak concentrations near birth after which CA II levels decrease while CA IV continues to increase (Carter et al. 1990; Lonnerholm and Wistrand 1982; Fleming et al. 1993). It is interesting to speculate that the rapid postnatal expression of CA IV (Fleming et al. 1993) may be associated with the beginning of air breathing and its appropriate regulation. Acetazolamide reduces fetal lamb lung fluid and H^+ secretion 30–65% (Adamson and Waxman 1976; Davis et al. 1980, 1989). The clinical impact of reduced lung fluid secretion and acidification on survival and growth, however, is unknown, but it possibly may not be crucial since CA II deficient mice appear to have no obvious pulmonary hypoplasia or difficulty at birth in making the transition to air breathing.

Active fluid reabsorption is necessary to counter passive fluid fluxes across the alveolar capillary membrane and to promote efficient gas exchange. Despite the switch from fluid secretion in utero to fluid reabsorption ex utero, the small amount of alveolar lining fluid remains acidic with micropuncture measurements of pH measured between 6.2 and 6.9 (Effros and Chinard 1969; Nielson 1986). The mechanism of acid secretion in the adult lung is uncertain. It may simply be a consequence of greater active sodium uptake relative to chloride (Effros et al. 1989) and a fall in pH on that basis, or to direct H^+ secretion by type II pneumocytes (Lubman et al. 1989) possibly related to the protons secreted into surfactant containing granules (Chander et al. 1986). The purpose of acid secretion may be to enhance the surfactant's surface tension lowering properties (Wildeboer-Venema 1984), to enhance intraacinar collateral ventilation pathways (Traystman et al. 1978; Swenson et al. 1998), or to magnify the pH changes in the parenchymal extracellular space with changes in PCO_2 that may serve as V_A/Q matching signals (see below). It would be predicted given the role of CA in fluid secretion and reabsorption in other epithelia that CA inhibition should decrease alveolar fluid reabsorption, but Chen et al. (2008) found in an isolated rat lung model that acetazolamide and methazolamide did not alter fluid reabsorption during hypercapnia. However, they did not study reabsorption under normal acid–base conditions.

Like many organs the lung is capable of defending its intracellular pH (pHi) against acidic and alkaline stresses (Wood and Schaefer 1978; Lubman and Crandall 1992). pHi control may be important for surfactant synthesis and the rate at which either H^+ or HCO_3 can be supplied or dissipated by Na^+/H^+ antiport, H^+ATPase, $C1^-/HCO_3$ exchange and Na^+/HCO_3 symport (Lubman and Crandall 1992) may be important in a number of metabolic pathways. CA inhibition slows the rate of pHi correction in alveolar epithelial cells (Heming et al. 1991) and the rates at which lung tissue stores or releases CO_2 with a change in alveolar PCO_2 (PIewes et al. 1976).

3.5.3 *Ventilation-Perfusion Matching*

The efficiency of the lung in gas exchange arises from effective matching of regional alveolar ventilation (V_A) and perfusion (Q). However, even at rest, regional blood flow and ventilation are not constant and may fluctuate 10–20% over intervals as short as one min (Swenson et al. 1998). When blood flow or ventilation change in a region, the alveolar PO_2 and PCO_2 will change accordingly. If these fluctuations in blood flow or ventilation are not quickly matched by corresponding changes in the other flow, V_A/Q mismatch is created. Several mechanisms in the lung evoke rapid responses in one flow to a change in the other including hypoxic pulmonary vasoconstriction (HPV) and pH dependent changes in airway and vascular smooth muscle tone (Swenson et al. 1998). Thus more rapid translation of the pH change arising from a change in local PCO will accelerate pH-mediated compensatory responses. Both HPV (Swenson et al., 1998) and hypocapnic bronchoconstriction and pneumoconstriction (Swenson et al. 1995) are slowed by CA inhibition with the half-time response increasing from roughly 50 to 100 s. When acetazolamide was given to normal dogs or to dogs with an imposed regional perfusion fluctuation, V_A/Q mismatching was greater and arterial oxygenation worse after CA inhibition (Swenson et al. 1993, 1995). The deterioration *of* V_A/Q matching with acetazolamide cannot be accounted for the associated systemic acid–base effects of CA inhibition.

Airway fluid and bronchial regulation: The presence of CA in the airways has been given relatively little attention. It could possibly subserve fluid secretory or absorptive functions as well as pH regulation. The pH of airway fluid is acidic (pH 6.6–7.0) in many but not all mammalian species (Smith and Welsh 1993; Robinson et al. 1989; Gatto 1981; Jack et al. 1990). Smith and Welsh (1994) and Devor et al. (1999) showed that cultured human lower airway epithelia actively secrete protons or HCO_3 depending upon the stimulating conditions. Bicarbonate-dependent chloride secretion is also dependent on CA (Cuthbert et al., 2003; Krouse et al. 2004). These regulated functions may provide an optimal lining fluid for mucociliary function and host defense in the upper respiratory tract (Boucher 1994). Cavaliere et al. (1996) found that dichlorphenamide increased the pH of human nasal secretions. Steel et al. (1994) and Devor et al. (1999) showed that 100 uM acetazolamide reduced the short circuit current of both human and sheep airway epithelium. At higher concentrations, acetazolamide (1 mM) combined with bumetanide reduced all anion secretion in serous glands and caused ductal mucus impaction (Inglis et al. 1997). As noted above, by indirect measurements, Thornell et al. (2018) concluded that airway lining liquid has minimal CA activity provided by membrane apical enzyme and propose that this absence helps to maintain a stable pH against the swings in airway PCO_2 during inspiration and expiration. CA XII in the airway epithelium appears to support normal chloride secretion, and its absence leads to symptoms and signs akin to cystic fibrosis (Lee et al. 2016a, b).

Certain diuretics, including furosemide, bumetanide, and chlorothiazide, inhibit non-allergic bronchoconstriction (Elwood et al. 1991). Dose response studies with loop diuretics of differing potency against the Na^+-K^+-$2Cl^-$ cotransporter suggest

that the bronchoprotective action of these drugs is not via Na-CI-K cotransporter inhibition (O'Connor et al. 1991) nor by Na" channel blockade (O'Connor et al. 1994). Common to all of these diuretics is that they are unsubstituted sulfonamides and as such have CA inhibiting activity. Therefore, inhibition of CA has been proposed to be the relevant property. Indeed, inhaled acetazolamide does block bronchoconstriction in mild asthmatics to cold dry air hyperventilation (O'Donnell et al. 1992), inhaled sodium metabisulfite (O'Connor et al. 1994) and cough induced by hypotonic aerosol inhalation (Foresi et al. 1996). However, the direct bronchodilating action of these drugs in non-constricted airways is not very potent (Barnikol and Diether 1979; O'Connor et al. 1994; O'Donnell et al. 1992).

The locus of action of CA inhibitors and inhaled diuretics on cough and bronchospasm are not known, but are thought to act on airway neural transmission (Elwood et al. 1991) since the enzyme has not been reported in bronchial smooth muscle. Since CA is found in peripheral muscle afferent nerves (Riley et al. 1984; Szabolcs et al. 1989) and acetazolamide reduces contractile neuropeptide release from sensory nerve endings in the airways (Sun et al. 1993), it is proposed that CA inhibition in afferent airway nerves reduces the ability to depolarize and initiate bronchoconstriction in response to an irritant. However, Verlcden et al. (1994) showed that 10^{-4}M acetazolamide did not alter electric field stimulation-induced cholinergic contraction in human airways. Whether CA is involved in airway neurotransmission is debatable since all the studies cited above except that of Verlceden et al. (1994), the concentrations of acetazolamide generally exceeded $10^{-3]}$ M, a concentration at which many non-specific effects of sulfonamides not related to CA inhibition occur (Maren 1977), and in no cases were proper dose response studies performed.

3.5.4 Pleural Fluid Composition and Turnover

Despite lack of biochemical or cytochemical evidence of CA in the pleural epithelium, it appears that the enzyme may be involved in the generation of an alkaline pleural fluid (Rolf and Travis 1971). Zocchi et al. (1991) found that pleural fluid reabsorption was reduced by 30% and had a lower bicarbonate when 100 uM acetazolamide was added to the pleural space. Results with anion and cation transport blockers suggest that CA subserves operation of a Na^+/H^+ and Cl^-/HCO_3 double exchange mechanism on the serosal aspect of the parietal pleura.

3.6 Carbonic Anhydrase Inhibitors and Cardiovascular Disease

3.6.1 Systemic Hypertension

Hypertension is a major factor in coronary heart disease, sudden death, stroke, and congestive heart failure due to the chronic mechanical stress placed on the heart and vasculature (Hollander 1976). Thus, treatment of essential hypertension has become one of the most critical interventions to decrease cardiovascular morbidity and mortality. Treatment of hypertension begins with lifestyle modifications, such as smoking cessation, weight loss, exercise, and cardioprotective diets, but most patients will require pharmacotherapy, using a combination of multiple medications like thiazide-type diuretics, calcium channel blockers, and angiotensin-converting enzyme inhibitors/angiotensin receptor blockers (Ferdinand and Nasser 2017; Taddei 2015).

The use of CA inhibitors in hypertension treatment dates to the release of acetazolamide in treating heart failure. By enhancing the urinary elimination of Na^+ and Cl^-, along with bicarbonate and potassium, sulfonamide CA inhibitors, acetazolamide, ACTZ, and ethoxzolamide, ETZ, reduced elevated blood pressures in patients with congestive heart failure (Relman et al. 1954, Moyer and Ford 1958; Moyer and Hughes 1995; Schwartz et al. 1955) The diuretic effect in these patients results from the inhibition of CA II, IV, XII, and XIV in both the proximal and distal tubule and is likely the most important antihypertensive mechanism. However, with the introduction of furosemide and other more potent loop diuretics, the use of CA inhibitors soon fell out of favor.

Other sites of action of the CA inhibitors on systemic blood pressure include vascular smooth muscle and endothelial cells. When studied in individual organ circulations in vivo and in isolated preparations, much work finds vessel relaxation with CA inhibitors. Likewise, in non-vascular smooth muscle sites, acetazolamide inhibits agonist-mediated ileal and vas deferens constriction (Carmignani et al. 1981), and neural-mediated bronchoconstriction (Elwood et al. 1993). Any consideration of CA inhibition in the vasculature must first recognize that both vascular endothelial cell and the smooth muscle CAs will be inhibited. Lacking studies in isolated cells of either type except in the pulmonary circulation (see below) any change in blood flow or vessel resistance may be the result of CA inhibition in one cell type or both.

The vascular endothelium generates local vasoconstrictors and dilators in response to a variety of stimuli, including shear stress, hypoxia, hypercapnia, and circulating hormones. The transduction and intracellular signalling pathways are complicated and involve numerous involve ion channels, some of which are pH sensitive (Nilius and Droogmans 2001; Taylor et al. 2006). Endothelial cell generated NO is of considerable interest in this regard. CA IV and other plasma-facing membrane bound isozymes easily accessed by drugs in the blood are associated with caveolae (Ryan et al. 1982); regions of the endothelial cell plasma membrane involved in vasoactive mediator binding and signal transduction, which highly express endothelial nitric

oxide synthase (eNOS). In the retinal circulation, vasodilation with dorzolamide is blocked by inhibiting eNOS-mediated NO production (Kringelholt et al. 2012), but in the cerebral circulation, the vasodilating effect of acetazolamide is independent of NO (Kiss et al. 1999). Thus, not all circulations may share this feature of linkage to NO. One possibility proposed is that the CA can generate NO from nitrite and that this is enhanced by several CA inhibitors (Aamand et al. 2009). Two fundamental problems arising from this study are how CA might have a reductase activity given its active site or how ligating the zinc by these drugs in the active site of the enzyme increases this activity. Several recent studies including in vitro and in vivo studies have not have not been able to establish CA-mediated NO generation from nitrite (Andring et al. 2018; Pickerodt et al. 2019; Rosenbaek et al. 2018).

CA inhibition increases cerebral and choroid plexus blood flow (Kiss et al. 1999; Taki et al. 2001; Grossman and Koeberle 2000). Acetazolamide in rats (at 4 mg/kg) increases blood flow in liver, brain, and kidney, but not in the stomach and skeletal muscle (Taki et al. 2001). In the eye, a variety of CA inhibitors (acetazolamide, brinzolamide, and dorzolamide) all induce ciliary and retinal vasorelaxation (Kringelholt et al. 2012; Torring et al. 2009) to improve retinal oxygenation (Pederson et al. 2005). Ethoxzolamide, acetazolamide, and benzolamide, block norepinephrine-mediated mesenteric artery constriction in a dose–response manner and in nM concentrations consistent with their inhibitory potency against CA and diffusibility into tissue (Pickkers et al. 1999). Furthermore, they showed that extracellular acidosis generated by CA inhibitors in vivo is not critical in vasorelaxation because the vessels were studied in vitro under fixed acid–base conditions. In follow-up human studies, acetazolamide is vasodilating when infused into the forearm (Pickkers et al. 2001). This experimental model has the advantage that within the time frame of the experiment and amount of drug given, there were no systemic effects that might have secondarily altered local vascular tone. However, in these experiments CA inhibitors are only vasodilating at much higher concentrations (uM) and appear to do by inducing membrane hyperpolarization via activation of calcium-activated potassium channels (Pickkers et al. 2001). Whether these drugs bind directly to potassium channels or their activity is altered secondarily by changes in intracellular pH caused by acetazolamide in vascular smooth muscle is not known. In skeletal muscle the evidence suggests they bind directly, and their action is not dependent on CA inhibition since isolated membranes were studied and concentrations needed to alter channel activity were in the uM range (Tricarico et al. 2004). Another potential mechanism of CA inhibitor-induced membrane hyperpolarization is via blockade of membrane voltage gated calcium channels but again at concentrations well above that necessary to inhibit CA (McNaughton et al. 2004). These findings in aggregate suggest a complex picture of both CA-dependent and non-independent effect of these drugs on vascular tone and that any claims made for these drugs relating to CA inhibition must be critically cognizant of the dosing and concentrations employed and whether it is by endothelial or smooth muscle effects.

Any blood pressure changes with CA inhibition will be a summation of changes in extracellular fluid (ECF) volume, cardiac output, neuro-humoral responses, acid–base status, and direct drug-induced vasodilation. Because all CA inhibitors are

diuretics and cause a 5–10% reduction in ECF volume (Brechue et al. 1990), this alone should lead to a reduction in blood pressure. Despite mild volume depletion, many studies in normotensive humans treated with acetazolamide in the usual range of 2–5 mg/kg, sufficient to cause diuresis and a mild metabolic acidosis find no blood pressure reduction at rest or with exercise (Jonk et al. 2007; Swenson and Maren 1978). A few trials of acetazolamide in essential hypertension or renal disease were largely disappointing with only <10% of patients responding with meaningful pressure reduction (Brest et al. 1961; Megibow et al. 1948; Horita et al. 2006). The ineffectiveness of acetazolamide and far greater efficacy and lower side effect profile of the thiazides and other emerging classes of antihypertensive drugs in the 1950s and 1960s, very quickly extinguished any interest in CA inhibitors for hypertension treatment.

The only instances acetazolamide convincingly reduces systemic blood pressure are at high altitude (Parati et al. 2013), in sleep disordered breathing (Eskandari et al. 2018) and in idiopathic intracranial hypertension (Wall et al. 2014). The dosing at high altitude was in the low range (2–4 mg/kg) and this reduced the slight degree of systemic hypertension from hypoxia-mediated sympathetic activation that develops in persons otherwise normotensive at low altitude.

One more possible benefit to acetazolamide at altitude is the better preservation of subendocardial oxygenation (Slavi et al. 2013), which would be of advantage particularly to patients with coronary artery disease who travel to high altitude. In sleep apnea, the improvement in sleep quality and reduction in sympathetic nervous activation like at high altitude best explains the antihypertensive effect. In idiopathic intracranial hypertension, doses used were much higher (20–60 mg/kg) to achieve reductions in cerebrospinal fluid production and it cannot be ruled out that the weight loss and other factors related to widespread CA inhibition at these doses and greater side effects were not also involved.

This disappointing failure of CA inhibition to be more broadly useful in altering blood pressure using acetazolamide and other potent CA inhibitors suggests that any direct vasodilation (as discussed above) to cause blood pressure reduction may opposed by counter-regulatory responses or consequences of CA inhibition occurring elsewhere in the body. One important response to CA inhibition-induced volume depletion and metabolic acidosis is a compensatory activation of the sympathetic nervous system. Plasma norepinephrine at rest is elevated roughly two-fold in individuals taking acetazolamide suggesting sympathetic activation in response (Goldsmith et al. 1990). The same degree of sympathetic activation was noted with breathing 4% CO_2 which evokes an equal fall in arterial pH (0.08 units) as with acetazolamide. Interestingly at high altitude, the ventilatory response to hypoxia which causes arterial hypocapnia acts to keep blood pH higher than otherwise develops at low altitudes with CA inhibitors and so may explain an effective blood pressure reduction with acetazolamide (Parati et al. 2013). In addition, plasma renin and urinary aldosterone increase with acetazolamide (Favre and Vallotton 1984). Chronic use of acetazolamide and other CA inhibitors causes several mild to distressing side effects in about one third of patients that is not fully explained by the metabolic acidosis caused by renal CA inhibition (Swenson 2014a, b). These likely arise from non-specific effects

of neuronal and GI tract enzyme inhibition. Lastly, because there is no evidence for vasodilation in skeletal muscle (Taki et al. 2001), which constitutes over 60% of total body mass, this may contribute to the lack of in vivo blood pressure reduction. Whether correction of the metabolic acidosis with supplemental bicarbonate or citrate as has been done to reduce other side effects of CA inhibitors would reveal more blood pressure reduction has not been tested.

3.6.2 Cardiac Hypertrophy and Heart Failure

Cardiac hypertrophy is a major predictor of developing of heart failure, arrhythmia, and sudden death (de Simone et al. 2008). The heart responds to increased work and/or loss of myocardium with hypertrophic growth of existing cardiomyocytes to enhance pump function and reduce altered ventricular wall tension. This hypertrophic growth is compensatory in the initial stages, but as time progresses it becomes maladaptive and leads to diastolic heart failure (Maillet et al. 2013; van Berlo et al. 2013). Heart failure treatment often requires a combination of beta adrenergic, renin-angiotensin, and aldosterone blockade. Ventricular hypertrophy/failure and the augmented gene and protein expression of the CA isoforms, CA II, IV, and XIV may be induced by the common mechanism of ventricular stretch arising from an increased ventricular workload (Alvarez et al. 2013; Torella et al. 2014). There is also an increase in the hypoxia inducible factor-1 responsive isoform of CA (CA IX) in the pathophysiological response of the failing heart to ischemia (Holotnakova et al. 2008).

Inhibition of these CA isozymes by ETZ and BZ in a rat model of cardiac hypertrophy caused by a chronic coronary artery occlusion leads to decreased pulmonary edema, less LV enlargement and remodeling over a period of several months of treatment (Vargas et al. 2016). The hypertrophic response in ischemic heart disease is in part driven by increased sympathetic nervous activation and catecholamine release within the heart and circulation, dilation and over-stretch of cardiomyocytes, and stimulation of cardiac membrane NHE-1 (sodium-hydrogen exchanger), AE-1 (chloride-bicarbonate exchanger) and NBC (sodium bicarbonate cotransporter). Activation of all these membrane transporters, which non-covalently associate with CA isozymes to form metabolons (a close assembly of various proteins serving a single function), leads to intracellular alkalization, which raises intracellular cytosolic calcium concentration and initiates the generation of a number of hypertrophic signals. By partially blocking the activity of these alkalinizing transporters by inhibition of their supporting CAs and causing a slight degree of intracellular CO_2 retention, hypertrophy can be reduced, and cardiac contractile function improved.

In addition to the benefit from CA inhibitors in reducing pathological cardiac hypertrophy, CA inhibitors can contribute to the prevention or reduction of pulmonary and systemic edema in congestive heart failure through their actions in the kidney to block the reabsorption of Na^+ and causing diuresis and natriuresis. It was this first indication for acetazolamide that markedly improved the treatment of

patients with heart failure in the 1950s (Leaf et al. 1954; Relman et al. 1954; Moyer and Hughes 1995), before the advent of current loop diuretics, such as furosemide. Another more potent CA inhibitor, ETZ) was also clinically used as diuretic agent in patients with heart failure (Moyer and Ford 1958). Two other compounds, chlorothiazide and hydrochlorothiazide, which show CA inhibitory properties and diuretic effects have been used for treatment of patients with congestive heart failure and post-hypertensive cardiac insufficiency. These thiazides, like furosemide and other related loop diuretics are considerably weaker CA inhibitors by several orders of magnitude. In the usual dosing for these transporters, Na–K–2Cl cotransporter in the loop of Henle and the epithelial Na channel in the distal nephron, respectively, they do not achieve concentrations high enough to be effective against renal CAs. The CA inhibitors differ from the loop diuretics and distally acting diuretics in causing bicarbonate loss and metabolic acidosis in contrast to enhanced distal tubular hydrogen ion excretion and metabolic alkalosis.

High-dose loop diuretic therapy is the primary cause of metabolic alkalosis in pediatric and adult patients with heart disease. ACTZ has been safely used in many patients with heart disease to lower serum pH and HCO_3, raise Cl^- and correct the metabolic alkalosis of chronic loop diuretic therapy (Moffett et al. 2007; Lopez et al. 2016; Wongboonsin et al. 2019). Acetazolamide can also improve the natriuresis of loop diuretic treatment (Imiela and Budaj 2017; Verbrugge et al. 2019). In June 2018, a multicenter, randomized study, (ADVOR-Acetazolamide in decompensated heart failure with volume overload) was initiated to assess the role of acetazolamide in combination with loop diuretics to limit loop diuretic resistance and metabolic alkalosis to better improve the outcomes of acute heart failure with volume overload (Mullens et al. 2018). Interestingly, the cardiovascular benefits of CA inhibitors may mimic the benefits seen with the introduction of the sodium-glucose cotransport inhibitors on cardiovascular outcomes in patients with diabetes beyond their effects to lower blood glucose by blocking reuptake of glucose in the proximal tubule and promoting natriuresis (Leon-Jimenez et al. 2018).

Another possible benefit of ACTZ in patients with chronic heart failure is reduction of exercise-induced periodic breathing and central sleep apnea which may compromise ventilation and oxygenation (Apostolo et al. 2014; Wongboonsin et al. 2019) in part by caused by loop diuretic induced metabolic alkalosis. Heart failure also leads to myocardial edema. In a mouse study of heart failure, acetazolamide treatment reduced myocardial water content, in part by inhibition and down-regulation of myocardial aquaporin-1 expression (Song et al. 2018).

3.6.3 Coronary Artery Disease and Myocardial Infarction

In experimental animal settings, CA inhibitors benefit the acutely ischemic heart. Benzolamide and ethoxzolamide slow the efflux of H^+ from the isolated buffer-perfused ferret heart and thus recovery of pH_i following a ten minute period of ischemia (Vandenberg et al. 1996). The drugs only very minimally slowed the

recovery of left ventricular developed pressure (LVDP) during reperfusion. In a series of subsequent experiments in the isolated rat heart using longer ischemic times of 60–90 min, benzolamide reduced infarct size by almost 80%, attenuated pathological hypercontracture, and improved post-ischemic recovery of myocardial contractile function (Ciocci-Pardo et al. 2018). The cardioprotective benefits of BZ in terms of reducing infarct size and improving contractility are likely the result of prolongation of the acidic conditions during early reperfusion, since 10 min of initial reperfusion in the same experimental model with a perfusate of 6.4 prevents cardiomyocyte damage occurring during reperfusion. This phenomenon of acidic protection during reperfusion has been termed the pH paradox and has been described in a number of organ ischemia-reperfusion models. In the acidic milieu, the otherwise tissue damaging effects of radical oxygen species and inflammatory cytokines generated when oxygen is returned are blunted and unnecessary apoptosis is limited. The protection afforded by benzolamide is best explained by its inhibition of membrane-bound CAs that enhance the function of membrane acid–base transporting proteins (NHE-1, AE-3, and NBC) to rapidly export the acid accumulated and take up bicarbonate. The protection by benzolamide is dependent upon p38MAP kinase-dependent pathways (Ciocci-Pardo, 2018) and by endothelial nitric oxide synthase upregulated NO production (Gonzalez Arbelaez et al. 2018). These dramatic findings along with those of CA inhibition in chronic heart failure offer a new possible strategy for treating patients with acute and chronic coronary artery disease, if replicated in human studies.

Another potential advantage to acetazolamide administration in acute ST segment myocardial infarction is that it can protect against radiographic contrast induced kidney injury at the time of percutaneous coronary artery stenting, when contrast must be given to visualize the blocked coronary arteries (Assadi 2006; Pakfetrat et al. 2009).

3.7 Carbonic Anhydrase Inhibitors in Pulmonary Disease

3.7.1 Chronic Obstructive Pulmonary Disease (COPD)

Within the decade of its introduction, clinicians began to explore whether acetazolamide might be a useful respiratory stimulant for hypoxemic patients with chronic obstructive pulmonary disease (COPD) by the increase in ventilation which occurs with a mild metabolic acidosis generated by renal CA inhibition with the goal of improving arterial oxygenation (McNicol and Pride 1961). Although it was effective in this regard for some patients with mild or moderate lung function impairment, many with moderate or severe disease could not tolerate the worsened dyspnea when forced to breathe more. In some cases, the drug led to hypercapnic respiratory failure (McNicol and Pride 1961; Schwartz et al. 1955). While the increased work of breathing with hyperventilation is trivial in healthy persons, in those with limited lung function and weaker chronically fatigued respiratory muscles the added

effort may not be possible or sustainable. Following a brief period of enthusiasm for use in COPD, this approach with few exceptions has been largely abandoned.

Seven studies in stable patients with hypercapnic COPD have shown that acetazolamide at 250–500 mg dosing increases arterial PO_2 by roughly 4–8 mmHg and decreases pH by 0.04–0.07, $PaCO_2$ by 3–7 mmHg and HCO_3 by 6–9 mM (reviewed in Adamson and Swenson 2017). Five of these seven were randomized placebo-controlled studies and all gave detailed pulmonary function data as well as exclusion criteria, most notably renal disease and $FEV_1 < 500$ ml. Of critical importance to issues of safety, tolerability and effectiveness, mean FEV_1 across all these studies was 24 to 39% predicted and only a handful of patients had $FEV_1 < 20\%$ predicted. No study was longer than four weeks, and side effects and quality of life were not measured. There were no reductions in exacerbations, hospital admissions, or mortality. In one such study (Skatrud and Dempsey 1983) comparing acetazolamide with medroxyprogesterone, responders to drug therapy were identified as those sustaining a fall in $PaCO_2$ of >5 mmHg during treatment. Responders were also able to lower $PaCO_2$ by 5 mmHg during 30–60 s of voluntary hyperventilation. The non-responders (mean fall in $PaCO_2$ of 1 ± 1 mmHg) were characterized by a lower mean FEV_1 of 24% predicted versus 33% in the responders. This capacity to lower $PaCO_2$ by greater than 5 mmHg by voluntary hyperventilation is largely dependent on the ability to increase tidal volume rather than rate (Skatrud et al. 1980). These studies in stable outpatients demonstrate that acetazolamide can increase ventilation and improve arterial blood gas values in patients with mild to moderate COPD, but it may not be effective in very severe COPD. Recent work by Dominelli et al. (2018) has shown that even in healthy humans, acetazolamide in typical clinical dosing reduces both maximal diaphragmatic strength as well as peripheral skeletal muscle strength, results not found with methazolamide, findings confirming earlier work by Kiwull-Schöne et al. (2001) in the rabbit. Thus the improvements in PO_2 with acetazolamide are no better than either beginning or slightly increasing low flow supplemental oxygen, which has no side effects and does not reduce diaphragmatic muscle strength.

Similar to outpatients, studies in hospitalized patients show that acetazolamide improves arterial oxygenation and lowers HCO_3 equivalently when given intravenously in doses of 250–500 mg to mechanically ventilated patients with co-existing metabolic alkalosis (Swenson 1998; Adamson and Swenson 2017). In most studies, this also was associated with reductions in $PaCO_2$, but no change in pH as reductions in HCO_3 were generally matched by decreases in PCO_2. The intent in most of these studies was to correct or diminish a metabolic alkalosis considered to be hindering liberation from the ventilator, but the degree to which the co-existing metabolic alkalosis, either primary (from diuretics, steroids, gastric suctioning, hypokalemia or hypoproteinemia) or secondary (post-hypercapnic) caused true arterial alkalemia (i.e., pH > 7.45) was less than 5%.

Whether acetazolamide enhances weaning or alters other clinically relevant outcomes in COPD exacerbations, the limited literature is informative. In a double blind randomized placebo-controlled study of 70 patients with hypercapnic respiratory failure and coexisting metabolic alkalosis ($PaCO_2 > 53$ mmHg and base excess >

8 mM), but not requiring intubation, acetazolamide (250 mg twice daily) or placebo was given for five days (Gulsvik et al. 2013). In comparison to the control arm, PaO_2 improved by 4 mmHg more in the treated group, while $PaCO_2$, base excess, pH, and potassium fell. Although this study was not powered to study hospital length of stay, it was no different in the two groups. Forty percent of patients given acetazolamide complained of non-specific side effects. Three patients had to be withdrawn because their arterial pH fell below 7.30.

In a retrospective 1:1 pair-wide case–control study of 72 mechanically ventilated patients with COPD and hypercapnic respiratory failure, acetazolamide (250 mg twice daily) did not alter time on the ventilator (Bahloul et al. 2015). Most recently, a randomized, placebo-controlled, double-blind, multicenter study of 380 patients with COPD exacerbations requiring intubation was performed to study the primary outcome of the effect of acetazolamide on the duration of invasive mechanical ventilation (Faisy et al. 2016). Placebo or acetazolamide (500–1000 mg daily) was given for the first two days after intubation. No significant difference was found in the primary outcome, or in weaning duration or ICU length of stay, despite achieving a significant reduction in serum bicarbonate concentration in the treatment group. Again, as in many studies, the degree of metabolic alkalosis (HCO_3 26 $\pm$ 7 mM) was minor; none of the patients had arterial pHs >7.45, renal function was normal and baseline FEV_1 values were greater than one liter.

Thus, while these studies in mechanically ventilated patients demonstrate that acetazolamide can improve oxygenation, it does not reduce the duration of mechanical ventilation. This position is supported by two reviews (Bales and Timpe 2004; Jones and Greenstone 2001) and the US Food and Drug Administration has not approved the use of acetazolamide to hasten liberation of patients with COPD from mechanical ventilation.

3.7.2 Chronic Cough

Carbonic anhydrase is expressed in the airway mucosa and nerves (Hanson et al. 1981; Kumpulainen and Korhonen 1982) and in CO_2 sensitive receptors termed neuroepithelial bodies (Livermore et al. 2015; Domnik and Cutz 2011). Sensory nerves in the airways are intimately involved in cough responses (Lee and Yu 2014). Inhibition of airway CA by deposition of several inhaled CA inhibitors given as aerosols and achieving enzyme inhibiting concentrations, including acetazolamide, furosemide and hydrohclorothiazide, all suppress cough reflexes and irritant-mediated cough (Foresi et al. 1996; Elwood et al. 1993; Ventresca et al. 1990; Stone et al. 1993). Chronic cough caused by Pertussis infection in an animal model is reduced with acetazolamide (Scanlon et al. 2014). These findings all suggest a role of inhaled acetazolamide for chronic cough, which the first author has used successfully in several patients after extensive investigation could find no remediable cause of protracted cough.

3.7.3 Pulmonary Hypertension (PH)

More recent and on-going studies point to a possible role of CA inhibitors in the treatment of several forms of pulmonary hypertension. The first of these include several diseases with hypoxia as a primary cause—WHO Group III PH. Hypoxia at the alveolar level is a pulmonary vasoconstrictor. Pulmonary artery pressure rises at high altitude or in hypoxic lung diseases due to hypoxic pulmonary vasoconstriction (HPV) which is a normal response of the lung vasculature to low oxygen levels in the alveolar gas that cause the surrounding blood vessels to constrict (Swenson 2013). HPV can cause both an acute problem within days known as high altitude pulmonary edema (HAPE) in which the lungs become congested as a result of the higher pulmonary artery pressure and to a more chronic problem of fixed pulmonary hypertension. In the chronic situation the constant elevation of pulmonary artery pressure ultimately causes the right side of the heart to weaken and fail to pump enough blood to the left side of the heart to maintain an adequate cardiac output.

3.7.3.1 High Altitude Pulmonary Edema (HAPE)

HAPE is the sudden development of pulmonary edema in otherwise healthy persons who have ascended within one to five days to high altitude. It is caused by pressures high enough within the microvasculature and capillaries of the lung to overcome the structural integrity of the ultrathin alveolar capillary barrier that maintains a dry fluid-free airspace. This hydrostatic breach of the normal permeability leads to alveolar hemorrhage and fluid accumulation that prevent normal oxygen and carbon dioxide exchange. The edema causes even lower arterial oxygen levels than otherwise at that altitude, cough, breathlessness, fatigue, inability to do minimal exertion, and ultimately death if not treated (Bartsch and Swenson 2013). In some persons HPV is quite excessive and pressures can rise enough to lead to capillary stress failure (Bartsch and Swenson 2013). Drugs which lower pulmonary artery pressure, oxygen, and descent are used for HAPE treatment and certain of the same drugs used to treat HAPE can be used prophylactically to prevent its occurrence. Acetazolamide, in principle, should be beneficial in preventing HAPE since the ventilatory stimulation it induces will raise alveolar PO_2 and thus diminish the principle stimulus for HPV. Furthermore, its diuretic effect might lower the total amount of fluid available to leak into the lung. A third possible benefit is a direct action on the pulmonary vasculature.

On the basis of earlier studies into the role of CA in gas exchange, ventilation–perfusion heterogeneity and control of ventilation, it emerged that acetazolamide and other CA inhibitors might directly inhibit HPV and offer protection against HAPE. The first report of HPV inhibition by acetazolamide was buried in a report on the effects of hypercapnia on the isolated perfused lung (Emery et al. 1977). This novel and unprecedented finding of a CA inhibitor effect on a process not thought to involve acid–base exchange or a pH transduction signal went wholly unrecognized for more than a decade until CA mediation in the peripheral chemoreceptor response

to hypoxia was demonstrated (Iturriaga et al. 1991). To explore the question fully, my colleagues and I conducted work in isolated pulmonary artery smooth muscle cells, isolated perfused lungs and live animals. A comprehensive approach was necessary since CA is ubiquitously expressed throughout the body and consequences of CA inhibition might arise from direct pulmonary effects and/or secondary systemic effects.

The isolated perfused lung permits the study of HPV without the possible confounding effects of systemic hypercapnia and metabolic acidosis following CA inhibition in the whole animal, which in general are known to augment HPV. Acetazolamide (30 uM in the perfusate) reduced HPV by roughly 50% and reduced the rate of rise by 40% (Deem et al. 2000). Without providing any explanation for HPV inhibition, there was no rise in exhaled nitric oxide (NO) as a marker for increased NO production to account for HPV moderation.

Studies in the live animal are important to determine whether findings in the isolated perfused lung are reproducible in vivo, in which non-pulmonary effects of CA inhibition; systemic acidosis, effects on peripheral chemoreceptors, and neural transmission might potentiate or oppose HPV at the lung and vascular level. In unanethetized beagles (Hohne et al., 2004, 2007) with invasive monitoring of pulmonary hemodynamics, ventilation, lung gas exchange, and renal function, acetazolamide (20 mg/kg) to achieve an equivalent concentration in blood to that used in the isolated perfused lung completely inhibited HPV when the dogs breathed 10% oxygen gas (F_IO_2 of 0.10). Lowering the dose of acetazolamide to 5 mg/kg, intravenous or oral (a dosing more relevant to human use) in a series of preliminary experiments also inhibited HPV, but not to the complete extent as 20 mg/kg (Hohne et al. 2007; Pickerodt et al. 2014). Inhibition of HPV could not be correlated with changes in plasma potassium, endothelin 1, or angiotensin II; factors that themselves alter HPV. Furthermore, HPV suppression in the whole animal occurs despite the systemic acidosis with CA inhibition. In several subsequent human studies, HPV is reduced by as much as 50–70% (Teppema et al. 2007; Ke et al. 2013; Boulet et al. 2018).

Altogether the data in the isolated perfused lung and whole animal clearly established that CA could be involved in the full expression of HPV, what process(es) it subserves and what cell type(s) in the lung (alveolar epithelial, vascular endothelial or arterial smooth muscle) are relevant could not be resolved by these experiments. Although HPV is a complex process (Swenson 2013), it is an inherent property of the pulmonary arterial and venous smooth muscle cells. In rat pulmonary artery smooth muscle cells obtained from small to mid-sized resistance vessels, acetazolamide had no effect on intracellular calcium (Ca^{2+}) in normoxia, but the drug markedly slowed and reduced the magnitude of Ca^{2+} uptake upon exposure of these cells to 4% O_2 (Shimoda et al. 2007) with an I_{50} of roughly 50 uM. To explore the mechanism by which acetazolamide inhibits HPV, experiments showed that acetazolamide does not act by inhibiting voltage-gated Ca^{2+} channels, does not alter membrane potential, or cause significant intracellular pH changes.

It is a general rule in pharmacology that one should never base conclusions on a single drug or concentration and CA pharmacology is no exception (Maren 1977).

When two more potent CA inhibitors, benzolamide (a hydrophilic membrane impermeant inhibitor) and ethoxzolamide (a lipophilic cell membrane-permeant inhibitor), were studied in both the isolated smooth muscle cells and the unanethetized dog, neither of these more powerful CA inhibitors (Shimoda et al. 2007) had any effect on the intracellular rise in Ca^{2+} of hypoxic pulmonary artery smooth muscle cells and were equally ineffective in the conscious dog since neither inhibits HPV (Hohne et al. 2007). Finally, methylation of the sulfonamide nitrogen critical to the binding with zinc at the active site of the enzyme to yield an inactive drug, N-methyl acetazolamide (NMA), surprising showed in both the pulmonary artery smooth muscle cells and in the dog, that this drug with otherwise the exact same structure beyond the sulfonamide moiety, pK, water and lipid solubility, and intramolecular electronic charge distribution to acetazolamide, but no CA inhibiting activity was equipotent at inhibiting Ca^{2+} elevation with hypoxia and reducing HPV (Shimoda et al. 2007; Pickerodt et al. 2014). In the cell studies NMA did not lower intracellular pH, and in the dogs, it did not cause a diuresis, change urinary bicarbonate excretion, or stimulate ventilation—all the expected findings of CA inhibition.

It is clear that acetazolamide inhibits HPV at the level of the pulmonary artery smooth muscle. It does so by a mechanism not dependent upon carbonic anhydrase and is not substantially altered by the effects of inhibition of carbonic anhydrase elsewhere in the body. In this case, the response of the pulmonary vasculature interestingly differs from that of the systemic vasculature in which the evidence is more compelling for a CA mediated role in vasomotor regulation. It has been shown that isolated porcine mesenteric arteries pre-constricted with norepinephrine relaxed with acetazolamide but also to methazolamide and ethoxzolamide in a dose response manner consistent with their respective CA inhibitory potencies (Pickkers et al. 1999). Intra-arterial infusions of acetazolamide into the human forearm reduce local vascular resistance and the response can be blocked by an inhibitor of Ca^{2+} activated K^+ channels (Pickkers et al. 2001). The molecular receptor for acetazolamide involved in HPV remains unknown.

As to whether acetazolamide itself prevents HAPE, it does so in a rat model in which 20 mg/kg prevents the typical alveolar protein, red cell, and lung water accumulation typical of human HAPE when rats are exposed to one-half atmosphere (~18,000 feet) for 24 h (Berg et al. 2004a, b). In humans the only supportive data are anecdotal unpublished reports by physicians in the mountains of Colorado who use acetazolamide to prevent re-entry HAPE in children returning home to high altitude after long holidays at sea level. A placebo controlled randomized study is presently underway to determine if acetazolamide or congeners of acetazolamide are efficacious. Use of a non-CA inhibiting form of acetazolamide if proven effective would be superior to acetazolamide in that the many side effects of CA inhibition could be avoided.

3.7.3.2 High-Altitude Pulmonary Hypertension

There has been very little work done in residents of high altitude who have pulmonary hypertension exploring whether acetazolamide or other CA inhibitors lower pulmonary artery pressures. The best studies have been performed in high altitude natives of the South American Andes, who develop a unique condition called chronic mountain sickness (CMS). CMS is characterized by greater erythrocytosis or polycythemia than that expected for the altitude of residence. By consensus, CMS is a hemoglobin concentration >21 g/dl in men and >18 in women. This degree of polycythemia causes increased blood viscosity, pulmonary hypertension, worse arterial oxygenation, thromboembolism, reduced cardiac output and work capacity along with a relative degree of hypoventilation compared to the higher ventilation of healthy high altitude residents. In both animal studies modeling CMS (Pinchon et al. 2012) and three studies in patients with CMS, acetazolamide lowers pulmonary artery pressure and hemoglobin concentration, increases ventilation and arterial oxygenation, and improves exercise capacity and quality of life (Richalet et al. 2005, 2008; Sharma et al. 2017). These improvements are indicative of a multifactorial effect of acetazolamide acting favourably on erythropoiesis, hemorheology, cardiac output, ventilation and tissue oxygenation. The limitation of the use of acetazolamide for CMS arises from the fact that the areas of the world where CMS is most prevalent do not have extensive health care systems to cover the costs of chronic treatment even with relatively low cost non-patent protected drugs.

Elsewhere in the world, the Himalayan and the Rocky Mountains, high altitude pulmonary hypertension in the absence of excessive erythrocytosis is just beginning to come under study with various drugs used to treat pulmonary hypertension at low altitude. Faoro et al. (2007) studied humans after ten days at 4,700 m and found that oral acetazolamide for one day at 250 mg tid did not reduce pulmonary artery (PA) pressures at rest or during exercise. Similarly, Basnyat et al. (2008) found that acetazolamide also did not reduce PA pressure in trekkers when started many days after reaching a high altitude start site (4,250 m). To date, however, acetazolamide or other CA inhibitors have not been tested except in rat and mice models. In mice and rat hypoxia models, it appears that hypoxia up-regulates the expression of aquaporin-1 (which is blocked by acetazolamide as discussed above) and knockdown of aquaporin-1 by different means limits hypoxic pulmonary hypertension (Schuoler et al. 2017; Yun et al. 2017; Liu et al. 2019). Very recently, acetazolamide in the same mouse model of (Schuoler et al. 2017) was efficacious in reducing aquaporin-1 expression and reducing pulmonary hypertension (Haider et al. 2019 submitted for publication). In an inflammatory/hypoxia pulmonary hypertension created by the combination of a vascular endothelial growth factor inhibitor in combination with ambient hypoxia, acetazolamide was effective in reducing pulmonary artery pressure. It did so by reducing inflammation possibly by inhibition of alveolar macrophage CA II, which is expressed at high concentrations in the hypertensive rats and in patients with pulmonary artery hypertension (Hudalla et al. 2019) or by the metabolic acidosis arising from renal CA inhibition (Christou et al. 2019). The only human studies suggestive of a salutary effect on a form of hypoxic pulmonary hypertension have

been in patients with sleep apnea treated with acetazolamide (Thurnheer et al. 2017; Ulrich et al. 2015). Given the improved ventilation with sleep and the resultant higher mean alveolar PO_2, a major contributor to lowered pulmonary artery pressure and vascular resistance will be the reduction in sleep disordered breathing, but a direct effect on the pulmonary vasculature remains a compelling possibility. Thus, acetazolamide as a treatment for high altitude pulmonary hypertension is worthy of greater investigation.

3.7.3.3 Non-hypoxic Pulmonary Hypertension

Pulmonary hypertension often develops in patients with congestive heart failure and depressed left ventricular diastolic and systolic function as a result of chronic pulmonary venous hypertension and propagation of the elevated pressure to the pulmonary arteries. The early studies of acetazolamide mentioned above in heart failure included some with cor pulmonale, or right heart failure due to left heart failure. In those studies, cor pulmonale was improved. The improvements were certainly due to diuresis and reduction in peripheral and pulmonary edema, but direct effects on the pulmonary vasculature were never studied in those days due to the lack of now widely available flow directed pulmonary artery catheters and more recently echocardiography. Another possible benefit already shown in the heart of reduced pathological remodeling of the left ventricle (Vargas et al. 2016) could be the same in the right ventricle. Despite mounting evidence in several forms of hypoxic pulmonary hypertension, there have been no studies of the other many causes of pulmonary hypertension in patients with CA inhibitors. Presently there is a large placebo controlled trial of acute and chronic acetazolamide treatment in patients with various forms of non-hypoxic pulmonary hypertension underway in Switzerland (Ulrich and colleagues, personal communication).

3.7.4 Lung Ischemia–Reperfusion Injury

Similar to positive results in moderating ischemia-reperfusion injury in the heart as described above (Ciocci-Pardo et al. 2018; Gonzalez Arbelaez et al. 2018), brain (Di Cesare Mannelli et al. 2016), liver (Bejaoui et al. 2015), and kidney (An et al. 2013), two studies in the lung have shown excellent preservation of structure and function, reduction in inflammation and apoptosis if acetazolamide is given just before the return of perfusion. In the first (Lan et al. 2017), the dosing of acetazolamide in a rat model was very high 100–400 mg/kg, dosing not practical or tolerable in humans. The second studied lower doses (30 mg/kg) of acetazolamide, benzolamide, and n-methyl acetazolamide (an analog of acetazolamide unable to inhibit CA) and found reductions in injury by all three drugs. The findings in this study (Kumar and Swenson unpublished results) suggest that acetazolamide acts both by CA inhibition

and perhaps through its suppression of hypoxic calcium signaling, a non-CA dependent process (Shimoda et al. 2007). The approximate rate of lung ischemia-perfusion injury in human lung transplantation is such that 10–20% of all patients suffer early graft dysfunction requiring longer periods of mechanical ventilation and extended post-operative periods in intensive care.

3.8 Conclusions

Acetazolamide and other CA inhibitors have multiple actions involving both inhibition of CA and of other processes independent of CA that give foundation and promise to a greater role of these drugs in the treatment of many cardiovascular and pulmonary diseases. Under certain circumstances, such as in hypoxic situations and high sympathetic nervous system activation, CA inhibitors act as good antihypertensives in both the systemic and pulmonary circulations. They also appear to alter pathological left ventricular hypertrophic remodeling in ischemic coronary artery disease, and this may equally apply to diseases leading to cor pulmonale involving the right side of the heart. At high altitude, reduction of hypoxic pulmonary vasoconstriction may be useful in preventing high altitude pulmonary edema. Lastly, the protection afforded by acetazolamide and other CA inhibitors in many animal models of acute organ ischemia-reperfusion injury involving the brain, kidney, liver, heart, and lung offers the possibility of using these safe and clinically approved drugs in myocardial infarction and stroke, and in surgical procedures involving long durations of ischemia, such as is necessary in organ transplantation. Work for the future should be aimed at moving appropriately to well-designed clinical trials and in animal studies seeking to better understand the myriad ways, particularly for acetazolamide, of how this class of drugs work in such a variety of conditions.

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