Chapter 18
Carbonic Anhydrase Inhibitors and High Altitude Illnesses

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Abstract Carbonic anhydrase (CA) inhibitors, particularly acetazolamide, have been used at high altitude for decades to prevent or reduce acute mountain sickness (AMS), a syndrome of symptomatic intolerance to altitude characterized by headache, nausea, fatigue, anorexia and poor sleep. Principally CA inhibitors act to further augment ventilation over and above that stimulated by the hypoxia of high altitude by virtue of renal and endothelial cell CA inhibition which oppose the hypocapnic alkalosis resulting from the hypoxic ventilatory response (HVR), which acts to limit the full expression of the HVR. The result is even greater arterial oxygenation than that driven by hypoxia alone and greater altitude tolerance. The severity of several additional diseases of high attitude may also be reduced by acetazolamide, including high altitude cerebral edema (HACE), high altitude pulmonary edema (HAPE) and chronic mountain sickness (CMS), both by its CA-inhibiting action as described above, but also by more recently discovered non-CA inhibiting actions, that seem almost unique to this prototypical CA inhibitor and are of most relevance to HAPE. This chapter will relate the history of CA inhibitor use at high altitude, discuss what tissues and organs containing carbonic anhydrase play a role in adaptation and maladaptation to high altitude, explore the role of the enzyme and its inhibition at those sites for the prevention and/or treatment of the four major forms of illness at high altitude.

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1 Introduction

The initial use of a carbonic anhydrase (CA) inhibitor, acetazolamide, for high altitude acclimatization dates back to the 1960s; however, the underlying rationale was already evident in the 1930s with the initial experience arising from the use of sulfanilamide, the first effective oral antibiotic and serendipitously the first carbonic anhydrase inhibitor. This chapter will relate that history, discuss what tissues and organs containing carbonic anhydrase play a role in adaptation and maladaptation to high altitude, explore the role of the enzyme and its inhibition at those sites for the prevention and/or treatment of the four major forms of illness at high altitude; acute mountain sickness (AMS), high altitude cerebral edema (HACE), high altitude pulmonary edema (HAPE), and chronic mountain sickness (CMS). Most of what we understand about carbonic anhydrase inhibitors derives from cell, animal and human studies with acetazolamide and the results are interpreted as the consequence of CA inhibition. However, like any medication, CA inhibiting sulfonamides including acetazolamide may have effects separate from CA inhibition and evidence is emerging that these actions, either with or without concomitant CA inhibition may be useful at high altitude and in diseases associated with hypoxia and ischemia.

2 Acute Mountain Sickness and High Altitude Cerebral Edema

AMS is a constellation of symptoms experienced by many people in the first several days at high altitude. Although hypobaria accompanies any ascent to high altitude, poor acclimatization to acute high altitude is primarily intolerance to hypoxia, which manifests itself as headache, nausea, anorexia, gastrointestinal distress, poor sleep, generalized malaise and lassitude. At above 10,000 ft or 3,000 m, roughly 25–50 % percent of newcomers can be afflicted. AMS is not life-threatening and generally resolves spontaneously after several days. Very severe AMS, however, may evolve into HACE which can be lethal if not recognized and treated immediately. HACE is marked by brain swelling and increased intracranial pressure (ICP), which cause confusion, ataxia, convulsions and ultimately death.

Despite much investigation over the past 4 decades since it was first shown that acetazolamide is effective in AMS, our understanding of both the pathogenesis of AMS/HACE [1] and the actions of acetazolamide in these condition remains incomplete and more complicated than generally taught [2, 3]. This should come as no surprise considering the presence of carbonic anhydrase in many tissues relevant

to high-altitude adaptation or maladaptation, and to the presence of enzyme there and elsewhere, the inhibition of which may contribute to the non-trivial incidence of side effects, some of which mimic the symptoms of AMS.

AMS is thought to be a consequence of non-lethal cerebral hypoxia and compensations invoked to return cerebral oxygen delivery to a more satisfactory level, such as by cerebral vasodilation and increase in cerebral blood flow (CBF). Some critical level of hypoxia differing among individuals (perhaps based upon genetic predisposition) may lead to changes in cerebrovascular permeability and in the integrity of the blood brain barrier (BBB). Increased CBF in association with changes in BBB permeability and impaired CBF autoregulation lead to mild vasogenic interstitial edema; with a possible increase in intracranial pressure that may be perceived as headache and the other symptoms of AMS. Although slight brain swelling (about 1 % increase in brain water) does occur in AMS it does not correlate with symptoms and persons with no AMS symptoms have equal swelling as those without AMS, making it unlikely that this small amount of swelling is pathogenic. Another theory for AMS is that cerebral hypoxia causes an increase in radical oxygen species (ROS) generation [4] which can cause irritation of pain fibers in the trigeminal nerve and increase BBB permeability for small molecules with irritant effects on neuronal function. Prophylactic strategies and treatments for AMS have aimed to increase cerebral oxygenation, reduce fluid accumulation, and decrease ROS generation. HACE is very likely the most severe expression of AMS, in which there is unequivocal brain swelling, breakdown of the blood brain barrier, and bleeding into some areas of the brain [5]. CA inhibitors act or may act positively on all of these factors in AMS, and possibly in preventing HACE by limiting the progression of AMS to HACE.

A brief history of CA and its inhibitors serves to give background perspective on acetazolamide use in AMS. Shortly after the discovery of CA in red blood cells in 1932 sulfanilamide, the first non-toxic oral antibiotic was introduced [6]. Almost immediately it was evident that animals and patients taking the drug develop a mild diuresis, a mild metabolic acidosis, and hyperventilation. These side effects were quickly recognized to be a consequence of renal CA inhibition. Following World War II, synthesis of stronger CA inhibitors yielded the 1,000-fold more potent sulfonamide, acetazolamide, which remains even to this day, the most commonly prescribed oral CA inhibitor. Although first used as a diuretic in heart failure patients and as a gastric acid suppressant, by the mid-1950s it found far greater efficacy in hydrocephalus and glaucoma, reducing both cerebrospinal fluid (CSF) and aqueous humor formation by 50 %. As an outgrowth of efforts to develop even more potent CA-inhibiting sulfonamides than acetazolamide, several other diuretics (high ceiling loop diuretics and the thiazides) acting on other ion transporters in the kidney were discovered. These have largely supplanted acetazolamide in diuretic therapy for heart and renal disease, unless a problematic metabolic alkalosis and alkalemia is present, for which the suppression of renal tubular bicarbonate reabsorption by acetazolamide helps to normalize acid–base status. Furosemide (and its analogs) and the thiazides are, in fact, weaker CA inhibitors, but equal or stronger in their diuretic action with fewer side effects, including no metabolic acidosis. Over the

subsequent decades other uses of CA inhibitors have been identified for diseases in which some degree of fluid transport or acid–base alteration might be advantageous, including epilepsy, macular edema, sleep disordered breathing and other primary hypoventilation syndromes, obesity, and now most excitingly cancer.

Within the decade of its introduction, respiratory physiologists and clinicians began to explore whether acetazolamide-induced metabolic acidosis might be a useful respiratory stimulant for hypoxemic patients with chronic obstructive pulmonary disease (COPD) with the goal of improving arterial oxygenation [3]. 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 and in some cases the drug led to hypercapnic respiratory failure [3, 7]. 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. In fact, the United States Food and Drug Administration lists severe COPD as a possible contraindication to its use.

Kronenberg and Cain, nonetheless, realized that such ventilatory stimulation might have a significant impact at high altitude in healthy persons, who can easily increase their breathing to significantly raise arterial PO_2 and blood oxygen content [8]. Indeed they showed better oxygenation and ventilation in subjects taking acetazolamide (5–10 mg/kg) undergoing simulation of high altitude in a hypobaric chamber. Shortly thereafter, Forwand et al. [9] demonstrated in a field study that this ventilatory stimulation by acetazolamide could reduce AMS symptoms, and its use for this purpose was quickly established and embraced. Since the 1970s over 200 studies with acetazolamide have shown it to be safe and 60–80 % effective in AMS, but still associated with side effects that some people cannot tolerate. It is interesting that some of the side effects experienced by those taking the drug at sea level (and thus not hypoxia-mediated) are some of the same symptoms of AMS- nausea, malaise, and loss of appetite [10]. Due to the overlap of side effects of acetazolamide with AMS symptoms, it may be that the effectiveness of acetazolamide in AMS is in fact underestimated.

Although an initial study of exercise in acute hypoxia found that acetazolamide taken for 1 day increased maximal oxygen consumption and work capacity by about 8 % [11], all subsequent studies, whether performed with acute hypoxia at sea level or after several days at high altitude showed either no improvement [12] or a slight decrease of about 5–10 % [13, 14]. Time to exhaustion at a high equivalent work rate also appeared to be slightly shorter after acetazolamide in two studies [12, 15, 16]. In all cases, the drug increases ventilation by about 10–15 % over control values and improves arterial oxygen saturation or arterial PO_2. Interestingly lactic acidosis (measured by elevation in blood lactate) is less with acetazolamide [17, 18] due not to decreased production but by decreased elimination out of muscle. In all the aforementioned studies the dosing of acetazolamide was of short duration (hours

to 1 day). Why maximal exercise capacity appears to be slightly compromised with acetazolamide at high altitude despite better arterial oxygenation could represent the higher metabolic cost of increased ventilation that limits cardiac output distribution to the exercising muscle and/or the greater intramuscular accumulation of lactic acid. In contrast, chronic administration of acetazolamide at high altitude causes less decrease in near-maximal O_2 consumption than in placebo control subjects [19] and less total weight and muscle mass loss, suggesting that overall better oxygenation both at rest and during exertion over many days helps to preserve muscle mass and power output. One more possible benefit to acetazolamide at altitude is the better preservation of subendocardial oxygenation [20], which would be of advantage particularly to patients with coronary artery disease who travel to high altitude.

3 Mechanisms of Action by Acetazolamide and Other CA Inhibitors

3.1 Renal Carbonic Anhydrase Inhibition

Acetazolamide works by several actions most of which relate to CA inhibition, but there may be others independent of the enzyme inhibition. The single and most important action is inhibition of renal proximal and distal tubular CA which leads to a loss of bicarbonate in the urine and generation of a mild metabolic acidosis [2, 6]. In response to the limited oxygen availability of high altitude, ventilation is stimulated by the hypoxia sensitive peripheral chemoreceptors, located in the carotid bodies, which project information to the respiratory control centers in the brain stem. However, as a consequence of stimulated ventilation, arterial PCO_2 falls and blood pH rises and the resultant respiratory alkalosis limits the full ventilatory response to hypoxia by reducing input from the peripheral and central chemoreceptors, both of which are suppressed by hypocapnia. During the next several days at altitude, the kidneys respond by reducing bicarbonate reabsorption to partially counteract the respiratory alkalosis, in effect reversing the braking action of hypocapnia on the hypoxic drive to breathe. Thus in essence, acetazolamide at doses of 1–10 mg/kg simply accelerates the normal response of the kidneys that otherwise requires several days to occur once at high altitude. It is important to note that owing to the organic acid concentrating capacity of the kidney, these low doses achieve virtually complete CA inhibition in the kidney.

Another possible benefit to renal CA inhibition is the mild diuretic and natriuretic effect of acetazolamide. Although it is not exactly known if fluid retention precedes and contributes to AMS or is simply a consequence of AMS, it is conceivable that a preemptive loss of extracellular volume might be beneficial. This remains speculative and studies of other mild diuretics (without CA inhibiting effect) such as spironolactone have not been conclusive [21, 22].

3.2 Vascular Endothelial Cell CA Inhibition

The other tissue CA activity which may be fully inhibited at this low dose range is that of the vascular endothelium throughout the body, but most importantly in the brain. Here membrane bound isoforms of CA, such as CA IV and XII, with their extracellular orientation can be easily inhibited at low drug concentrations. Luminal brain vascular endothelial CA will be fully and immediately inhibited by even very low drug concentrations in blood, causing a small hindrance to normal "tissue-to-blood" transfer such that tissue PCO_2 will be elevated by 1–2 mmHg [23]. A slight CO_2 retention in the vicinity of both the central and peripheral chemoreceptors by this inhibition in combination with the renal metabolic acidosis will be sufficient to stimulate ventilation. Both cause elevations in H^+ concentration and counteract the effect of increased ventilation to washout CO_2 and raise pH. Acetazolamide (5–7 mg/kg iv) in normoxic humans induces a small increase in ventilation within minutes consistent with endothelial CA inhibition, well before any significant urinary bicarbonate loss occurs or red blood cell drug uptake reaches a critical inhibitory level [24]. At the level of the central chemoreceptors in the brain stem, this slight retention of CO_2 leads to greater efferent signaling to the respiratory control centers in the brain stem. While it might be logical to assume that red cell CA inhibition might also contribute to the efficacy of acetazolamide, it does not appear that sufficient red cell CA inhibition occurs at the low doses effective in AMS. Although inhibition of red cell carbonic anhydrase does cause ventilatory stimulation, the combination of high erythrocyte CA concentrations and in humans, two isozymes (CA I and CA II) requires much greater dosing (>15 mg/kg) to cause sufficient CO_2 retention and respiratory acidosis to stimulate ventilation [25].

3.3 CA Inhibition in the Central Nervous System

In the brain, three sites of CA might be involved in the protective action of acetazolamide; choroid plexus, cerebral vasculature and chemoreceptors. The choroid plexus secretes CSF and any reduction in CSF production will help to diminish intracranial pressure (ICP), which is set by the amount of water in the blood and interstitial fluid volumes, intracellular volume and CSF volume. In healthy conditions these volumes, although contained within the rigid confines of the skull are such that ICP is less than 20 mmHg. Yet, any increase in one or more of the component cranial volumes can quickly elevate ICP to values that begin to limit blood flow and or cause pain and neurological alterations. Complete choroid plexus CA inhibition reduces CSF production by 50 % and in principle could reduce overall intracranial volume and pressure in AMS. However, effective doses of acetazolamide which penetrate the BBB to reach the choroid plexus, and depress CSF flow are on the order of 20 mg/kg [26] and much greater than doses effective in AMS.

At the level of the cerebral vasculature, acetazolamide in high concentrations (>20 mg/kg) causes vasodilation, increases CBF and oxygenation [27], but at the lower doses used successfully in AMS, there is no increase in CBF [28, 29]. In fact, one study using 250 mg every 8 h for three doses found a slight fall of 10 % at simulated altitude, but enhanced autoregulation [29]. However, these changes were not correlated with AMS symptoms. Despite the lack of CBF increase or even slight fall, cerebral oxygenation was 3–5 % higher at rest and exercise in trekkers between 3,700 and 5,700 m while taking 375 mg bid [30]. These increases in cerebral oxygenation were greater than the corresponding improvements in arterial oxygenation generally observed with acetazolamide. Whether acetazolamide reduces cerebral oxygen consumption and so by this mechanim also increases cerebral oxygenation remains unexplored. The only caveat in wholly dismissing a possible benefit of increased CBF is that if regional brain blood flow changes are heterogeneous, then measurements of total CBF may not reflect increases in blood flow in some critical areas of the brain whose oxygenation will be improved by greater perfusion. There have been no studies of whether low dose acetazolamide alters regional CBF when total CBF is not increased.

The final site of CA in the central nervous system is the chemoreceptors, located in the brain stem (only CO_2 and pH sensitive) and the peripheral chemoreceptors of the carotid body (sensitive to O_2, as well as CO_2 and pH). Here it gets rather complicated in vivo, because the behavior and signaling of the two sites of chemoreception are clearly altered by systemic changes in acid–base status and oxygenation with high altitude, in addition to any possible consequence directly of their own enzyme inhibition. When the central chemoreceptors are studied in isolation it appears that the speed at which they respond to changes in PCO_2 or pH are slowed, but not the full response when CA is inhibited [31]. If local metabolically produced CO_2 is not as efficiently eliminated by loss of neurocellular and endothelial cell CA activity the slight tissue CO_2 retention will further decrease intracellular pH and stimulate ventilation in addition to the metabolic acidosis caused by renal CA inhibition.

When the peripheral chemoreceptors are studied in isolation inhibition of CA by methazolamide leads to a slowing of the response to a change in PCO_2 or pH, and also a reduction in the maximum response [32, 33]. In contrast, methazolamide delays, but does not decrease the response to hypoxia [32]. With respect to changes in ventilation or carotid body output with hypoxia, there is no increase in the acute isocapnic hypoxic ventilatory response (HVR) in vivo despite a renal metabolic acidosis. This indicates that local chemoreceptor CA inhibition abolishes the additive H^+-O_2 interaction in the peripheral chemoreceptors [24, 34, 35]. A lower peripheral chemoreceptor response to hypoxia has also been demonstrated during exercise [36]. This raises an interesting question as to what could be the advantage of taking a drug at altitude that has inhibitory effects on oxygen-sensing cells. The answer lies in the fact that the ventilatory response to high-altitude hypoxia is under partial suppression by the hypocapnic alkalosis. Consequently, any agent abolishing the H^+-O_2 interaction under these circumstances will blunt the action

of a low PCO_2 on the hypoxic response and generate more ventilation than would otherwise occur [2, 3]. In humans at sea level, acetazolamide has no influence on the peripheral chemoreceptor contribution to the ventilation response on stepwise changes in end-tidal PCO_2 [37]. It cannot be excluded that, in part, H^+-O_2 and O_2 responses follow separate signal-transduction pathways in the carotid bodies. Future studies with acetazolamide are warranted to determine if, despite the absence of an H^+- O_2 interaction, the carotid bodies may retain their H^+ sensitivity. Predictably, the inhibitory effect of acetazolamide inhibition on HVR should be due to peripheral chemoreceptor CA inhibition. However, in the cat, even the more lipophilic inhibitor methazolamide does not reduce HVR [38], suggesting that acetazolamide and other CA inhibitors may act by a mechanism (s) other than CA inhibition as taken up below in the next section on HAPE.

The actions of acetazolamide on chemoreceptors and the vasculature suggest also how it reduces periodic breathing (PB) during sleep and in sleep apnea at high altitude [39, 40]. Repetitive apnea and hyperpnea leads to cyclical drops in arterial oxygenation that may disrupt sleep quality and add to the total hypoxic stress of high altitude. It has been proposed [41] with loss of higher cortical control on breathing during sleep that hypoxia-mediated high peripheral chemoreceptor output leads to a level of ventilation during sleep sometimes high enough to raise PaO_2 and lower $PaCO_2$ sufficiently to transiently suppress breathing. During such an apneic period, PaO_2 then falls and $PaCO_2$ rises enough to generate a combined strong stimulus to initiate overbreathing again which then repeats itself often 30–60 times an hour. During the hyper-ventilatory phase arterial PCO_2 may decrease enough to pass below the apneic threshold and exceed the "CO_2 reserve" (i.e., the difference in $PaCO_2$ during eupnea (normal breathing) and the apneic threshold). The CO_2 reserve when combined with "plant gain" (or the ventilatory increase required for a given decrease in $PaCO_2$) and "controller gain" (ventilatory responsiveness to CO_2 above eupnea), are the key determinants of breathing stability in sleep. In general, breathing during sleep is made more stable with increases in the CO_2 reserve, and reductions in plant and controller gain. In addition the rate at which the chemoreceptors respond with changes in their output to the respiratory control centers to varying and rapidly arterial O_2 and CO_2 levels may also be important. Acetazolamide alters all of these favorably. First, with elevated arterial PO_2 and decreased $PaCO_2$, plant gain will be reduced because larger changes in ventilation are needed to cause equivalent changes in blood gases. Second, a parallel left shift of the CO_2 response curve (ventilation vs. $PaCO_2$) will raise the difference between the prevailing $PaCO_2$ and the apneic threshold (CO_2 reserve) and decrease the propensity for apnea. A small rise in brain stem PCO_2 with inhibition of vascular CA will have a similar tonic stabilizing influence. Third, due to the higher prevailing PO_2, subjects are in a flatter region of the (hyperbolic) hypoxic response curve, resulting in a reduced O_2 controller gain. A further reason why acetazolamide reduces ventilatory controller gain in sleep is the abolishment of the CO_2-O_2 interaction so that responses to hypoventilation-induced combined hypoxia/hypercapnia may be reduced considerably. Lastly acetazolamide by slowing the rate at which the chemoreceptor afferent information arrives at the respiratory control centers

could help dampen the magnitude of the ventilatory responses and in particular keep the arterial PCO_2 from going below the apneic threshold. Finally, it cannot be ruled out that acetazolamide increases the cerebrovascular response to combined hypercapnia/hypoxia [18], leading to a dampening influence on subsequent changes in brain stem PCO_2. Note that a lowered cerebrovascular CO_2 sensitivity may play a role in the genesis of periodic breathing at high altitude [42].

3.4 Non-CA Inhibiting Actions

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

3.4.1 Aquaporin Modulation

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 (AQP) family, AQP-1 and AQP-4 are of potential interest with regard to acetazolamide and high altitude disease. In addition aquaporins may also serve as channels for small uncharged gas molecules, such as nitric oxide (NO) and CO_2 [43, 44]. Both AQP-1 and AQP-4 are expressed in the brain [45], particularly astrocytes and choroid plexus, where they are involved in CSF production [46], CBF [47], and brain extracellular fluid and water homeostasis [48]. Inhibition of AQP-4 and genetic deletion of AQP-4 is protective against some forms of cerebral edema [49, 50]. AQP-1 is the major isoform in red cells and the kidneys and intimately involved in whole body osmoregulation and possibly in CO_2/HCO_3 fluxes [51]. With regard to AQP-4 and to some extent AQP-1, some groups have found that acetazolamide in the uM range directly blocks water flux across the plasma membrane of cells in vitro [46, 52, 53]. In the study by Tanuimura et al. [53] interestingly, methazolamide was inactive, despite its very close structural similarity to acetazolamide; differing only by a methyl group substitution on the thiadiazole ring. Despite these several reports of AQP-1 and AQP-4 blockade by acetazolamide others have not been able to confirm these findings [54–56].

Although resolution of the question of whether acetazolamide directly blocks aquaporins is difficult, this does not necessarily rule out other means by which acetazolamide could alter brain water homeostasis involving aquaporin function. Acetazolamide has been reported to reduce vasogenic and cytotoxic forms of cerebral edema in animal models [57–61]. Although acetazolamide may reduce edema by other actions such as diuresis and vascular dilation, it also inhibits AQP-1 and AQP-4 gene and protein expression in models of brain and cardiac injury

[61, 62] and so by this action favorably alter brain water homeostasis. These actions of acetazolamide on cerebral edema and brain likely have less relevance in AMS than in HACE where frank edema occurs.

3.4.2 Radical Oxygen Species Modulation

The evidence that hypoxia causes increased radical oxygen species (ROS) generation, tissue injury or dysfunction, and vascular leakage is growing. Hypoxic exposure equivalent to typical high altitudes increases ROS formation [63, 64] and some studies show that AMS can be prevented by antioxidant administration [65]. If ultimately it is proven that increased ROS formation is responsible for AMS, then acetazolamide, a heterocyclic thiadiazole might perhaps work as an antioxidant given that other numerous compounds containing a 1-3-4 thiadiazole ring are ROS scavengers [66]. Intriguingly the depression of peripheral chemosensitivity to hypoxia by acetazolamide in humans can be overridden by antioxidants [67]. This somewhat paradoxical finding would suggest that acetazolamide may not be a ROS scavenger, but this remains to be tested directly. Natural defense against ROS includes a number of antioxidant proteins, such as superoxide dismutase, catalase, hemooxygenase and thioredoxins that are upregulated by the gene transcription factor, nuclear related factor-2 (Nrf-2). 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 [68]. Whether this difference between the drugs just represents the greater lipophilicity of methazolamide over acetazolamide and great penetrance across the blood brain barrier, or some unique attribute of methazolamide will require more extensive pharmacological investigation.

3.4.3 Heat Shock Protein (HSP), Interleukin-1 Receptor Agonist (IL-1RA) and Hypoxia-Inducible Factor (HIF)

Heat shock protein-70 protects against cellular stress induced by hypoxia and IL-1RA is an anti-inflammatory cytokine. In a human study, subjects who did not develop AMS had higher blood levels of HSP-70 that those with AMS and acetazolamide increased HSP-70 in the AMS susceptible subjects who did not get AMS or had fewer symptoms [69]. In this same study, acetazolamide also raised IL-1RA levels. It is not clear whether these interesting findings are due to CA inhibition or to other actions of the drug.

The master hypoxic transcription factor, HIF-1, appears important in surviving hypoxic stress. When normoxic rats were given exceedingly large doses of acetazolamide (50–100 mg/kg) HIF-1 alpha was upregulated in brain tissue along with several of the genes known to have HIF-1 response elements in their promoter region [70]. However, another study of similar degree of acidosis (pH $\sim$ 7.0) in cultured cells showed moderate up-regulation of HIF-1 protein, but interestingly

not all of the usual genes upregulated by HIF-1 with hypoxia were increased [71]. The relevance of these finding to high altitude is uncertain, because acetazolamide doses this high cause severe respiratory acidosis, but respiratory alkalosis occurs at high altitude. Whether the very slightly lower pH with acetazolamide at altitude compared to those not treated (usually about 0.05 units) causes any differences in HIF-1 metabolism remains unknown. Furthermore, two human studies have found no relationship between AMS susceptibility and numerous polymorphisms of HIF-1 [72, 73].

4 Clinical Aspects of Acetazolamide in AMS/HACE Prevention

Over 1800 subjects have been treated with acetazolamide in placebo-controlled trials and across all dosing regimens and altitudes examined the prophylactic efficacy of acetazolamide in preventing or reducing AMS severity is roughly 50–60 % [11, 74]. These meta-analyses found a slight dose response effectiveness increasing from 45 % with 125 mg bid to 50 % with 250 mg bid to 55 % with 375 mg bid. The calculated number of subjects needed to treat (NNT) to prevent one case of AMS is 7 with 125 mg bid, 6 with 250 mg bid and 3 with 375 mg bid. The only other CA inhibitors studied have been methazolamide and benzolamide. Methazolamide, a more lipophilic agent at equivalent CA inhibiting doses to acetazolamide appears to be equally effective [75, 76]. Benzolamide, with its ten-fold higher hydrophilicity and lower membrane permeance than acetazolamide was effective against AMS in a 72 h simulated altitude hypobaric chamber study [77] and in reducing periodic breathing during sleep at high altitude [78]. More recently, benzolamide at 100 mg bid was very effective at reducing AMS and maintaining better oxygenation in subjects in a Himalayan trekking expedition [79]. As treatment for AMS, one placebo controlled study of acetazolamide 250 mg bid showed that it hastened symptom relief and improved oxygenation within 24 h [80].

These studies of different CA inhibitors with varying intracellular penetrance (methazolamide > acetazolamide >> benzolamide) but similar efficacy in AMS prevention suggest strongly that the main impact of any CA inhibitor is by renal and endothelial cell CA inhibition, since benzolamide is largely excluded from the other sites of intracellular CA discussed above that might be involved in AMS pathogenesis. Because benzolamide [79] was found to reduce by roughly 50 % the side effects perceived by subjects and objective neuropsychological deficits (concentration, balance, memory recall) with acetazolamide 125 mg bid [81], it or similar impermeant inhibitors should be superior. Unfortunately, benzolamide has no patent protection and thus it is unlikely that any pharmaceutical company will market the drug. However, with an emerging realization that other plasma membrane-associated CA isozymes (IX and XII) are richly and almost uniquely expressed in cancer cells and critical to their growth and metastatic potential clinical development of other membrane-impermeant CA inhibitors which block CA IX are

underway and have shown promising features. These drugs would be ideal for use at high altitude. Until that time, the use of acetazolamide at doses at or below 125 mg bid may come close to yielding the same results as benzolamide, and is worthy of further study up to moderate altitudes and with slower ascent rates. Lastly, it must be appreciated that among individuals heterogeneity in body size, drug clearance rates, which may be slowed at high altitude [82] and as yet uncovered genetic susceptibility to AMS may make it necessary to use higher doses with greater ascent rates and maximal altitude goals.

Finally, whether acetazolamide can prevent or treat HACE remains untested and unknown. HACE is very rare and thus controlled trials will never be practical. Dexamethasone, a glucocorticoid effective in many forms of cerebral edema, works effectively at early stages of HACE and remains the treatment of choice. Although some physicians use acetazolamide with dexamethasone in HACE the evidence is all anecdotal. Given the threat of impending lethal intracranial pressure (ICP) elevation, higher doses (>20 mg/kg) capable of inhibiting CSF production and perhaps reduce AQP-4 to lower ICP should be considered rather than the conventional 2–4 mg/kg. This could be tested formally in a rat model of HACE recently reported that demonstrated effectiveness of a derivative of gingko biloba [83].

Many questions remain to be investigated before a complete understanding of acetazolamide protection in AMS and HACE is possible. Although not an exhaustive list of experiments and methodological approaches, the following are several key directions for future research.

1. Regional brain blood flow, pH, metabolic rate, and oxygenation (such as with magnetic resonance imaging and spectroscopy) in areas of brain involved in respiratory control need to be performed in humans in conjunction with ventilation, ventilatory responses, and arterial blood gas measurements. Dose–response experiments with acetazolamide in normoxia and hypoxia, combined with plasma measurements of total and free drug levels, are needed to better calculate the degree of red blood cell and tissue CA inhibition. Although not presently available for humans, analogs of acetazolamide, lacking CA inhibiting activity, will be useful in animals to test whether and how much of the effect of acetazolamide is due to CA inhibition, or to some other action on O_2 and CO_2 sensing and responsiveness, redox state, ion channels or oxygen- and oxygen radical-dependent transcription factors such as HIF-1 or Nrf-2.
2. Studies of acetazolamide in conscious and sleeping chemoreceptor- intact animals are needed, both in normoxia and hypoxia, in which central and peripheral chemoreceptor contributions can be gauged by isolating the drug and/or systemic acid–base changes to either the central or peripheral chemoreceptors. This can be done by isolating the circulation to the carotid bodies and perfusing them with appropriately conditioned blood [84].
3. The contributions of the renal metabolic acidosis and diuresis of acetazolamide to the protection with acetazolamide in AMS need to be explored by using other mild diuretics to achieve the same magnitude of diuresis as acetazolamide and/or using acetazolamide but preventing bicarbonate and sodium losses by carefully titrated replacement.

4. Studies of membrane-impermeant CA inhibitors would be extremely interesting and likely would show more efficacy and fewer side effects than acetazolamide, as the limited data on benzolamide demonstrate. These ought to tested when they become available and approved for human use in cancer treatment.

5 High Altitude Pulmonary Edema

Despite the overwhelming evidence of efficacy of acetazolamide in AMS over many decades of use, the logical extension to HAPE prevention and treatment has received no attention in all of the many studies of acetazolamide at high altitude.

HAPE is the sudden development of pulmonary edema in otherwise healthy persons who have ascended within 1–5 days to high altitude. It may also occur in high altitude residents that have descended for more than several days and then reascend to their usual altitude of residence (reentry HAPE). 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 barrier 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 [85]. Pulmonary artery pressure rises at high altitude due to hypoxic pulmonary vasoconstriction (HPV) and is a normal response of the lung to low oxygen levels in the alveolar gas that cause the surrounding blood vessels to constrict [86]. However, in some persons HPV is excessive and pressures can rise enough to lead to capillary stress failure [85, 87, 88]. 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.

On the basis of earlier studies into the role of CA in gas exchange, ventilation–perfusion heterogeneity and control of ventilation, I began to pursue the possibility that acetazolamide and other CA inhibitors might directly inhibit HPV and offer protection against HAPE. The first report of HPV inhibition by acetazolamide, however, was buried in a report on the effects of hypercapnia on the isolated perfused lung [89]. 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 [32].

My first inkling that CA might be involved in reducing HPV arose from studies with the multiple inert gas elimination technique (MIGET). We found that acetazolamide (20–25 mg/kg) increased ventilation–perfusion heterogeneity in

normoxic anesthetized dogs [90]. Using a global index of ventilation–perfusion (V/Q) mismatch (a summation of the alveolar-arterial differences for the six inert gases of MIGET), gas exchange efficiency decreased by roughly 25 %. We hypothesized that spontaneous minute to minute fluctuations of regional ventilation and/or perfusion lower or raise alveolar PCO_2 accordingly and evoke known pH-sensitive parenchymal, vascular, and airway bronchomotor responses to readjust the accompanying parallel gas or blood flow in a manner to return the local ventilation–perfusion ratio to its original state [90]. Since all of these CO_2 mediated changes are pH-dependent responses, slowing the rate of $CO_2/HCO_3\text{-}/H^+$ interconversion will reduce overall ventilation–perfusion matching. When we imposed known cyclic fluctuations in regional lobar perfusion of 60 s intervals to generate varying alveolar PCO_2 concentrations, overall ventilation–perfusion heterogeneity in dogs increased with acetazolamide [91], consistent with this hypothesis. Although our focus was on CO_2-pH sensitive mechanisms of V/Q matching in these studies, we reasoned that any fluctuation in regional ventilation or perfusion would, of course, also alter local alveolar PO_2 and thus the strength of hypoxic pulmonary vasoconstriction. In principle, our results were also consistent with a component of impairment and slowing of HPV by acetazolamide. It was this possibility that directed my focus to the pulmonary vasculature and the role, if any, of CA in pulmonary vascular smooth muscle physiology and hypoxic responses. In order 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.

6 Isolated Perfused Lung and Whole Animal Atudies

Our first studies used the isolated blood perfused rabbit lung, in which we measured both the rate of HPV development and its final magnitude [92]. The isolated perfused lung allowed us to examine 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 [86]. We found that acetazolamide (30 uM in the perfusate) reduced HPV by roughly 50 % and reduced the rate of rise by 40 % as earlier shown in the same preparation [89]. Without providing any explanation for HPV inhibition, we found no rise in exhaled nitric oxide (NO) as a marker for increased NO production to account for HPV moderation. Whether acetazolamide might act via enhanced vasodilating NO generation remains a subject of some controversy. Although we found no increase in exhaled NO as a surrogate for increased NO in the vasculature, others have reported carbonic anhydrase has nitrite reductase activity and that this CA-mediated NO generation from nitrite can be enhanced by acetazolamide and dorzolamide [93]. It is not at all clear how CA might have a reductase activity at its active site or how

ligating the zinc in the active site of the enzyme increases this activity. Because other hypoxia-sensitive nitrite reductases, such as xanthine oxidoreducatase and deoxyhemoglobin [94] generate more NO in the lung under hypoxic conditions, it is unlikely that acetazolamide works via increasing nitrite availability since renal CA inhibition leads to reduced nitrite reabsorption by the proximal tubule and greater urinary excretion [95].

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 [96] with invasive monitoring of pulmonary hemodynamics, ventilation, lung gas exchange, and renal function, we found that 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). Since conscious animals hyperventilate with CA inhibition (due to a combined respiratory and metabolic acidosis from red cell and renal CA inhibition) it was possible that the abolition of HPV may have resulted simply from the higher alveolar PO_2 with hyperventilation. In order to control for this potential reduced stimulus to HPV we lowered the F_IO_2 to 0.08 and achieved equivalent arterial and alveolar PO_2 as in the control animals breathing a F_IO_2 of 0.10. In this case, there was no difference in the effect on HPV. 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 [97]. We found in these experiments that 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.

7 Pulmonary Vascular Smooth Muscle Studies

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 [86] and is modulated by systemic acid–base status, peripheral chemoreceptor activity, the autonomic nervous system, and the pulmonary vascular endothelium, it is an inherent property of the pulmonary arterial and venous smooth muscle cells.

In the 60 years since its discovery CA has been found in virtually all cells including many in the lung; the alveolar epithelium, bronchial epithelium, vascular endothelium and in certain airway nerves [3]. However, up to this point it had never been demonstrated in the pulmonary vascular smooth muscle, although histochemical and immunocytochemical studies in other non-vascular smooth muscle sites

and systemic vascular smooth muscle clearly suggested its presence [98]. Using rat pulmonary artery smooth muscle cells obtained from small to mid-sized resistance vessels, we found that acetazolamide had no effect on intracellular calcium (Ca^{2+}) in normoxia, but markedly slowed and reduced the magnitude of Ca^{2+} uptake upon exposure of these cells to 4 % O_2 [99] with an I_{50} of roughly 50 uM. While one cannot directly translate these pharmacological data in isolated rat arterial smooth muscle cells to the isolated perfused rabbit lung and live dog, the I_{50} in our cell work accords well with the dose–response effects in these other studies. Thus, in the rabbit lung the concentration was 30 uM [92] and we only achieved partial inhibition, while in the dog the dose of 20 mg/kg yielding a concentration of approximately 70 uM [100] was fully inhibitory [96] and 5 mg/kg yielding a concentration of about 15 uM only partially blocked HPV [97].

To explore the mechanism by which acetazolamide inhibits HPV, we undertook a number of experiments with agents or manipulations that alter intracellular Ca^{2+} content in combination with acetazolamide and hypoxia . Whether the effect of acetazolamide on hypoxia-induced Ca^{2+} responses is due to a non-specific inhibition of Ca^{2+} signaling, we studied the effect of acetazolamide on the response to 60 mM extracellular KCl. Potassium is a non-specific vasoconstrictor that induces an increase in intracellular Ca^{2+} in pulmonary artery smooth muscle cells by initiating membrane depolarization and Ca^{2+} influx through voltage-gated Ca^{2+} channels. KCl caused a significant increase in intracellular Ca^{2+} which was similar in magnitude to that induced by hypoxia, but was unaffected by pretreatment with 100 uM acetazolamide. These results indicate that acetazolamide has no effect on the activation of voltage-gated Ca^{2+}channels and suggest that the effect of acetazolamide on pulmonary artery smooth muscle cell Ca^{2+} signaling during hypoxia is not due to toxicity or a non-specific or generalized inability to increase intracellular Ca^{2+} by initiating extracellular Ca^{2+} uptake [99].

Acetazolamide in neural and systemic vascular smooth muscle has recently been proposed to alter the properties of Ca^{2+} activated K^+ channels, causing hyperpolarization [101, 102]. Since the hypoxia-induced increase in intracellular Ca^{2+} is believed to occur secondary to depolarization, hyperpolarization should prevent activation of voltage-gated Ca^{2+} channels and an increase in intracellular Ca^{2+} in response to hypoxia. We measured membrane potential (Em) using an Em-sensitive fluorescent dye and found that acetazolamide had no effect on basal normoxic Em and did not alter the KCl-induced depolarization. Acetazolamide in normoxic cells caused a very small statistically significant decrease in resting intracellular pH (pHi) from 7.26 to 7.22. While intracellular pH changes are known to affect intracellular Ca^{2+} concentrations and so might conceivably alter HPV, in order to show intracellular Ca^{2+} changes equivalent to the 100–150 nM changes we observed with hypoxia [99], the intracellular pH needs to be altered by greater than 0.5 pH units [103]. Exposure to 4 % O_2 had no significant effect on pHi and did not further alter the pHi of cells pretreated with acetazolamide. Lastly, we have preliminary evidence that acetazolamide does not inhibit Rho-kinase and so does not act by altering Ca^{2+} sensitivity of the smooth muscle contractile apparatus or by reducing ROS formation caused by endothelin (unpublished data).

These results taken as a whole suggest that acetazolamide has a specific action on hypoxia-mediated Ca^{2+} increases that are not due to changes in pHi or membrane potential and membrane L-type Ca^{2+} channels. We are presently pursuing the possibility that acetazolamide may alter the hypoxic-mediated sarcoplasmic reticulum (SR) release of Ca^{2+}, which has been shown to initiate the much larger influx of Ca^{2+} from the extracellular space via plasma membrane surface store operated Ca^{2+} channels [104].

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 [105]. Thus, we tested two other more potent CA inhibitors, benzolamide (a hydrophilic membrane impermeant inhibitor) and ethoxzolamide (a lipophilic cell membrane-permeant inhibitor) in both the isolated smooth muscle cells and the unanethetized dog. To our complete surprise, neither of these more powerful CA inhibitors [99] had any effect on the intracellular rise in Ca^{2+} of hypoxic pulmonary artery smooth muscle cells although they did slightly lower pHi as did acetazolamide, indicative of CA inhibition which lowers pHi by impairing CO_2 diffusion out of cells. Likewise, we have shown these two inhibitors are equally ineffective in the conscious dog since neither inhibits HPV [97]. Finally we methylated 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). In both the pulmonary artery smooth muscle cells and in the dog, 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 [99, 106]. 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.

The relevance of the cellular and animal work has been established in humans. In a simulated 8 h exposure to 12 % oxygen, acetazolamide 250 mg bid given over 3 days reduced HPV by 80 % [35] and more recently 375 mg bid given 24 h before flying to 3,800 m also reduced pulmonary artery pressure over the next 2 days at high altitude [107]. These findings of acute effectiveness against HPV appear to be sustained for only several days at altitude or with hypoxic exposure because equivalent dosing in subjects at high altitude for more than 1–2 week does not lower pulmonary artery pressure [14, 108]. This suggests that over time other mechanisms resistant to acetazolamide such as smooth muscle hypertrophy causing pulmonary hypertension at altitude become more important in setting the elevated pulmonary vascular resistance than HPV.

At this juncture it is clearly apparent 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 preconstricted

with norepinephrine relaxed with acetazolamide but also to methazolamide and ethoxzolamide in a dose response manner consistent with their respective CA inhibitory potencies [109]. 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 [110]. The molecular receptor for acetazolamide involved in HPV remains unknown.

As to whether acetazolamide itself prevents HAPE, we have shown acetazolamide is effective [111] in an rat model in which 20 mg/kg prevents the typical alveolar protein, red cell and lung water accumulation of human HAPE when rats are exposed to one-half atmosphere (~18,000 ft) for 24 h. In humans the only supportive data are anecdotal unpublished reports by physicians in the mountains of Colorado who use acetazolamide to prevent reentry HAPE in children returning home from holidays at sea level. Placebo controlled studies are clearly needed to determine if acetazolamide or congeners of acetazolamide are efficacious and perhaps have been preventing many cases of HAPE concurrently with its use in AMS. 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.

8 Chronic Mountain Sickness

Presently more than 100 million people live permanently above 2,500 m in the Andean, Himalayan and North American Rocky Mountains. Most individuals are well adapted, particularly those descendants of many generations at high altitude. Nonetheless, some newcomers after weeks to months and resident natives develop excessive erythrocytosis or polycythemia beyond the normal slight increase in hematocrit and hemoglobin concentration which is a typical compensatory response to chronic hypoxia and high altitude residence. Hematocrits above 65 % cause increased blood viscosity and poor perfusion of all organs and tissues. Additionally the work of the heart is increased and leads to early cardiac dysfunction. Typical symptoms and findings of CMS include cyanosis, plethora, headache, fatigue, edema, systemic and pulmonary hypertension, relative hypoventilation and cor pulmonale (right heart failure). The pathogenesis of CMS and what drives excessive erythrocytosis are not known, but it is thought that either a hereditary lower drive to breathe (lower hypoxic and hypercapnic ventilatory responses) causes greater hypoxemia and more release from the kidneys of erythropoietin (EPO), the hormone that stimulates bone marrow production of red cells. Greater erythrocytosis leads to more hypoventilation and begets a vicious cycle [112]. Alternatively, the lower drive to breathe demonstrated in patients with CMS may be secondary to an initial extreme sensitivity of the kidney to hypoxia and greater EPO production that then causes secondary hypoventilation [113]. In either event, phlebotomy or drugs that stimulate breathing and secondarily suppress EPO release, or directly inhibit renal EPO production lower hematocrit, improve cardiac function and relieve symptoms of CMS [114].

Acetazolamide, by virtue of its ability to stimulate ventilation and prevent AMS has been explored as a low cost treatment option in CMS because phlebotomy is expensive and time consuming, while moving to low altitudes can be socially and economically disruptive. Two large randomized placebo-controlled studies performed in the Peruvian Andes found that chronic acetazolamide (250 mg daily) over 3 weeks or 6 months in patients with CMS and hematocrits above 65 % was quite effective and well tolerated. Roughly 40 % of patients had reductions in hematocrit below the definitional criterion for CMS, and there were improvements in arterial oxygenation, systemic and pulmonary blood pressures, CMS symptom scores, and reductions in EPO concentrations [115, 116]. Confirming the stimulation on ventilation, these authors found that both hypoxic and hypercapnic ventilatory responses were augmented with treatment [117]. In a rat model of CMS, acetazolamide either started before 3 weeks of hypobaric hypoxia to an equivalent of 5,500 m or started 10 days into the exposure reduced hematocrit, blood viscosity, pulmonary and systemic vascular resistance, and increased cardiac output [118, 119]. Relief of hypoxic pulmonary vasoconstriction by both an increase in alveolar PO_2 and a reduction in the stimulus for HPV as well as a direct inhibition of HPV [92, 96, 99] likely contribute to the higher cardiac outputs and amelioration of cor pulmonale.

The data and pathophysiological basis of CMS strongly support the concept that acetazolamide reduces EPO production by its ventilatory stimulation and it is unlikely that CA inhibitors work by any other mechanisms. However, it has been proposed that acetazolamide directly suppresses renal EPO production independent of improvements in oxygenation by decreasing the O_2 demands of sodium reabsorption in the proximal tubule where the majority of sodium bicarbonate reabsorption occurs and is inhibited almost 80 % by CA inhibitors [120]. In these normoxic rat experiments, the dosage of acetazolamide necessary to suppress EPO production was roughly 10–20 times higher than the dose yielding a natriuretic and bicarbonaturic effect. A better explanation for the suppressant effect of acetazolamide on EPO release at such high doses is the likely severe respiratory acidosis accompanying complete red cell CA inhibition [25] which is known to block normal EPO production [121].

9 Conclusions

Acetazolamide and other CA inhibitors have multiple actions that underlie their efficacy in the prevention of acute altitude illnesses. This is most clearly established for AMS, in which it is predominantly an effect of renal and vascular CA inhibition that generate a mild bicarbonaturia and slight tissue CO_2 retention in the central nervous system. These combined metabolic and respiratory acidoses counter the suppressive effects of the hypoxia-mediated hyperventilation-induced hypocapnic respiratory alkalemia, which acts to limit the full ventilatory response to hypoxia. These same actions also are protective against CMS. Actions of these inhibitors

on other CA-dependent processes such as CBF and CSF regulation may play a role as well, but experiments sufficient to isolate these effects from the ventilatory stimulation will require sophisticated 'clamping' of ventilation changes. Likewise, investigation of non CA-inhibiting actions of acetazolamide will also entail further work, such as has been performed in studies of hypoxic pulmonary vasoconstriction as they may relate to HAPE. In fact, the several non-CA inhibiting actions of acetazolamide warrant a reconsideration of much work that has used acetazolamide as the definitive pharmacological probe for defining a role for CA in a physiological process or mechanism as so cogently outlined by Thomas Maren in his classic review of the subject [105]. Lastly, definitive clinical trials of acetazolamide and non-CA inhibiting analogues of acetazolamide (such as n-methyl acetazolamide) in HAPE prevention are needed. With the advent of topical CA inhibitors for the treatment of glaucoma, such as dorzolamide, and the promise of isozyme specific CA inhibitors in cancer therapy, acetazolamide now has its greatest application at high altitude and remains the drug of choice for AMS and CMS prophylaxis and treatment.

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