Pharmacology of acute mountain sickness: old drugs and newer thinking

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Swenson ER. Pharmacology of acute mountain sickness: old drugs and newer thinking. J Appl Physiol 120: 204–215, 2016. First published August 20, 2015; doi:10.1152/japplphysiol.00443.2015.—Pharmacotherapy in acute mountain sickness (AMS) dates back to the first use of a carbonic anhydrase (CA) inhibitor, acetazolamide, in the 1960s for high-altitude acclimatization (41, 128). Corticosteroids, such as dexamethasone, used in the treatment of many forms of cerebral edema were shown efficacious in preventing and treating AMS by the mid-1980s (5, 66). These two drug classes are the mainstay of prevention and treatment, although other medications have been found effective, but in far fewer numbers of subjects. Those other drugs will not be discussed, except as they might relate to better understanding how CA inhibitors and glucocorticoids may work. CA inhibitors and glucocorticoids have very important other actions in addition to their classic effects with relevance to AMS. In this review, I discuss the numerous pathways that may be involved and exploited in the future with more selective drugs.

AMS is a symptom complex experienced by many in the first days at high altitude (13). It is primarily an intolerance of hypoxia, which manifests itself as headache, nausea, anorexia, gastrointestinal distress, poor sleep, generalized malaise, and lassitude. AMS is not life-threatening and generally resolves after 1-2 days. Very severe AMS, however, may progress to high-altitude cerebral edema (HACE), which is marked by brain swelling and increased intracranial pressure leading to drowsiness, confusion, stupor, ataxia, and ultimately death if not treated quickly (13).

The pathogenesis of AMS is the consequence of nonlethal cerebral hypoxia and likely compensations invoked to increase cerebral oxygen delivery. A critical level of hypoxia, based perhaps upon genetic predisposition, may lead to changes in cerebrovascular permeability and the integrity of the blood-brain barrier (BBB). Increased cerebral blood flow (CBF) in association with changes in BBB permeability and possibly 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. Slight global vasogenic extracellular edema (about 0.5–1.0% increase in brain water) occurs in AMS, but it does not correlate with symptoms, and persons without symptoms have equal brain swelling (5). A question remains as to whether those with the most severe AMS may in fact have increased intracellular swelling or cytotoxic edema (117). Another theory...
for AMS is that cerebral hypoxia causes an increase in radical oxygen species (ROS) generation (10), which can cause irritation of pain fibers in the trigeminal nerve (5) and increase BBB permeability (99). The headache and possibly the other symptoms of AMS may also be the result of hypoxic cerebral vasodilation, which by itself can trigger trigeminal nerve activation even in the absence of BBB changes or ROS production (112). However, most studies of subjects developing AMS do not find increased cerebral blood flow over that observed in subjects without AMS (2). Last, limitations in cerebral venous outflow present in some people may cause passive upstream vasodilation and engorgement (150). Prophylactic strategies and treatments for AMS have aimed to increase cerebral oxygenation, reduce fluid accumulation, and diminish ROS and inflammatory mediator generation. In addition, given the possibility of mild brain swelling and vascular engorgement in AMS noted earlier, strategies that modestly reduce cerebral blood volume might be useful.

ACETAZOLAMIDE AND OTHER CA INHIBITORS

Shortly after the advent in the 1930s of sulfanilamide, it was evident this first oral antibiotic caused a mild diuresis, 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. While acetazolamide failed as a safe and effective respiratory stimulant in patients with hypoxemic lung disease, it was realized that such ventilatory stimulation might be useful at high altitude in healthy persons, who can easily increase their breathing to significantly raise arterial Po2 and blood oxygen content (128). Since the late 1960s, over 200 studies with acetazolamide have shown it to be safe and 60–80% effective in AMS (74, 131), but associated with side effects that some people cannot tolerate. Interestingly, several 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. Due to the overlap of these side effects with AMS symptoms, it may be that the effectiveness of acetazolamide in AMS is underestimated (35, 131). Figure 1 provides a brief overview of the several actions of acetazolamide and other CA inhibitors that are taken up in detail in this section. While several actions of CA inhibitors independent of those that are hyperventilation induced are of considerable interest, this hyperventilation will remain the single best explanation for efficacy, because in essence it is equivalent to descending to a lower altitude.

ACTIONS AND MECHANISMS ATTRIBUTABLE TO CA INHIBITION

Renal CA Inhibition

The single important action of acetazolamide and other CA inhibitors is inhibition of renal tubular CA and subsequent loss of bicarbonate. This results in metabolic acidosis, reduced tubular reabsorption of sodium, increased urinary excretion of sodium, and increased diuresis (129). Acetazolamide reduces pH, bicarbonate, and NaHCO3 in the urine (129). The metabolic acidosis, diuresis, and hyperventilation that follow acetazolamide treatment lead to increased arterial PO2, reduced blood volume, and reduced cerebral blood flow (130). A diagram illustrating the many actions of acetazolamide and other CA inhibitors is presented in Figure 1.
of bicarbonate and generation of a mild metabolic acidosis (131). In response to the limited oxygen availability of high altitude, ventilation is stimulated by the peripheral chemoreceptors. However, increased ventilation causes arterial $P_{CO_2}$ to fall and blood pH to rise, such that the respiratory alkalosis acts as a brake on the full hypoxic ventilatory response. During the next several days, the kidneys respond by reducing bicarbonate reabsorption to partially counteract the respiratory alkalosis, in effect reversing the inhibitory action of hypocapnia on the hypoxic drive to breathe. In essence, acetazolamide at doses of 1–5 mg/kg simply accelerates the normal renal response that otherwise requires several days to occur. It is important to note that, owing to the organic acid concentrating capacity of the kidney, these quite low doses (~1 mg/kg) can achieve virtually complete and almost selective renal CA inhibition without effect on any other processes dependent upon the enzyme (128).

Another possible benefit to renal CA inhibition is the mild diuretic and natriuretic effect of acetazolamide, roughly causing about a 7% fall in extracellular and plasma volumes (131). Although it is not exactly known if fluid retention precedes and contributes to AMS or is simply a consequence, it is conceivable that a preemptive loss of extracellular and plasma volume might be beneficial, particularly as it might reduce total brain blood volume. The idea that diuresis may be beneficial in AMS remains speculative. Studies of other mild diuretics (without a CA-inhibiting effect) such as spironolactone have not been conclusive (14, 65, 80), as has also been the case with the much more potent diuretics, such as furosemide (3, 124). Furosemide is an extremely weak CA-inhibiting sulfonamide (131), and in its clinical dosing it does not inhibit renal CA, but rather causes a much larger diuretic effect by inhibiting the Na-K-2Cl transporter in the loop of Henle. The concern in using furosemide is that it may lead to considerable volume depletion and hypokalemia, a very hazardous situation in mountaineering or remote high-altitude locations. Furthermore, unlike acetazolamide, it is not a respiratory stimulant, and, by generating a metabolic alkalosis, it may depress the ventilatory response to hypoxia. Despite these two reports (3, 124), performed over 40 years ago, there have been no further studies and no published guidelines to advocate its use.

Vascular Endothelial Cell CA Inhibition

Another tissue CA activity which will be fully inhibited at the low-dose ranges used for AMS is that of the vascular endothelium in the brain and throughout the body. Membrane-bound isoforms of CA, with their extracellular orientation, can be easily and fully inhibited at low drug concentrations. This will cause a small hindrance to normal “tissue-to-blood” transfer such that tissue $P_{CO_2}$ will rise by 1–2 mmHg (76) in the vicinity of the central and peripheral chemoreceptors and the medullary respiratory control centers and will stimulate ventilation. Although inhibition of red blood cell CA causes ventilatory stimulation, the very high erythrocyte CA concentrations require much greater dosing (>15 mg/kg) to cause sufficient $CO_2$ retention and respiratory acidosis on this basis to stimulate ventilation (128).

CA Inhibition in the Central Nervous System

In the brain, three sites might be involved in the protective action of acetazolamide: choroid plexus, cerebral vascular smooth muscle, and chemoreceptors. Any reduction in cerebrospinal fluid (CSF) production by the choroid plexus can diminish intracranial pressure (ICP), which is set by the amount of water in the blood and interstitial fluid, and by intracellular and CSF volumes. Any increase in one or more of the component cranial compartments can quickly elevate ICP to values that begin to limit blood flow and cause pain and/or neurological sequelae. Complete choroid plexus CA inhibition reduces CSF production by 50% and could thus reduce overall intracranial volume and pressure in AMS. However, effective doses of acetazolamide which penetrate the BBB to reach the choroid plexus are on the order of 20 mg/kg (24), which are much greater than doses effective in AMS (74, 131).

In the cerebral vasculature, acetazolamide in high concentrations (as achieved with >20 mg/kg) causes vasodilation and increases CBF and oxygenation in normal and injured brains (52, 134). At lower doses used conventionally for AMS prevention, there is no increase in CBF with acetazolamide in normoxia (46) and even a slight fall in hypoxia as indicated by a 10% decrease in middle cerebral artery flow velocity (127). In one study, enhanced autoregulation was found (127), but was not correlated with AMS symptoms, as suggested by another study in which AMS scores were weakly correlated with impairment of autoregulation (143). Despite the lack of CBF increase, cerebral oxygenation was 3–5% higher at rest and exercise in trekkers between 3,700 and 5,700 m (145) and was greater than the corresponding improvements in arterial oxygenation generally observed with acetazolamide. Acetazolamide may also reduce cerebral oxygen consumption (147) and thus increase cerebral oxygenation. The only caveat in wholly dismissing a possible benefit of increased CBF is if regional brain blood flow changes are heterogeneous. Measurements of total CBF might not reflect increases in blood flow in critical areas of the brain where oxygenation is improved by greater perfusion and possibly better autoregulation. There have been no studies of whether low-dose acetazolamide increases blood flow in certain areas of the brain when total CBF is not increased, but with larger doses (>10 mg/kg) significant regional blood flow heterogeneities are observed (21, 140). There is no evidence that acetazolamide reduces global BBB permeability directly or as a result of its mild systemic acid-base alterations (97, 159). However, as with the case of possible critical heterogeneities in regional CBF differences, there may exist unrecognized differences in regional BBB characteristics, such as have been observed in other neurological conditions (22, 95).

The final site in the central nervous system (CNS) is the chemoreceptors, located in the brain stem and carotid bodies. 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 CA inhibition. When the central chemoreceptors are studied in isolation they respond to changes in $P_{CO_2}$ or pH more slowly (29). When the peripheral chemoreceptors are studied in isolation, inhibition of CA by methazolamide slows the response to a change in $P_{CO_2}$ or pH.
and also reduces the maximum response (64). With respect to changes in ventilation with hypoxia, there is no increase in the acute isocapnic hypoxic ventilatory response (HVR), despite a renal metabolic acidosis. This indicates that local chemoreceptor CA inhibition abolishes the additive H⁺-O₂ interaction in the peripheral chemoreceptors (139). The question arises: What could be the advantage of taking a drug at altitude that has inhibitory effects on oxygen-sensing cells? The answer may lie in the fact that the ventilatory response to high-altitude hypoxia occurs in the setting of an accompanying hypocapnic alkalosis. Consequently, any agent abolishing the H⁺-O₂ interaction under these conditions will blunt the action of a low Pco₂ on the hypoxic response and generate more ventilation than would otherwise occur (128, 137). Another possibility is suppression of periodic breathing (131); however, it is not clear that periodic breathing is a risk factor for AMS (37). Predictably, the inhibitory effect of acetazolamide on HVR should be due to peripheral chemoreceptor CA inhibition. However, even the more lipophilic inhibitor methazolamide does not reduce the magnitude of HVR (138), suggesting that acetazolamide and other CA inhibitors may act by a mechanism(s) other than CA inhibition, as shown in the pulmonary circulation (56, 57, 104). In those studies a single methyl group substitution on the unsubstituted sulfonamide amine group yielded a compound still active against hypoxic pulmonary vasoconstriction, but devoid of any CA inhibiting activity.

In summary, the benefit of acetazolamide and other CA inhibitors (e.g., methazolamide), acting as such in the doses studied and found effective, derive largely from renal and vascular CA inhibition and the resultant improvement in arterial oxygenation universally observed in every study. In this regard, the CA inhibitors differ from dexamethasone and other corticosteroids in that not all studies with dexamethasone find better arterial oxygenation when successfully used for AMS prophylaxis (see next section). Benzolamide, which by virtue of its very intracellular penetration due to its low oil/water partition coefficient and by its concentration in the kidneys and in the urine by tubular organic anion secretion, comes closest to inhibiting CA only at the kidneys and vascular endothelium (131). The limited studies with benzolamide, an orphan drug, show similar efficacy to acetazolamide (79). While physiological rationale can be made for benefits also stemming from reduction in CSF production by the choroid plexus and increases in CBF in AMS, the requirement for three to four times higher dosing than used in clinical trials of AMS makes it very unlikely that individuals might differ in the extent to which these processes could be clinically important. The opposing actions of CA inhibition in different tissues, e.g., vascular CA inhibition increasing ventilation in vivo and depression of hypoxic ventilatory response when CA inhibitors are given acutely before metabolic acidosis develops, highlights the difficulty of interpreting how CA inhibitors truly work. Selective tissue inhibition studies would be ideal, but, aside from benzolamide, there are no practical analogs available. When such drugs become available, it would be interesting to use them to understand fully the benefits of this class of drugs. Last, to what extent other actions of acetazolamide, independent of better arterial oxygenation (discussed later), are also salutary in AMS will require studies in which subjects taking acetazolamide are prevented from realizing higher arterial oxygenation as a result of their increased ventilation, e.g., by lowering the inspired oxygen to match the same Pao₂ they achieve while on placebo treatment.

** ACTIONS AND MECHANISMS UNRELATED TO CA INHIBITION**

As alluded to earlier, 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 at high altitude and in other diseases associated with hypoxia, edema, and ischemia.

**Aquaporin 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 trans epithelial fluid transport. Of the many members of the aquaporin (AQP) family, AQP-1 and AQP-4 are of particular interest with regard to acetazolamide and high-altitude disease. Both AQP-1 and AQP-4 are expressed in the brain (17), particularly in the choroid plexus and astrocytes, respectively, where they are involved in CSF production (17), CBF regulation (98), and brain extracellular fluid and water homeostasis (156). Inhibition of AQP-4 and genetic deletion of AQP-4 are protective against some forms of cerebral edema (27, 62, 120), and upregulation of these aquaporins causes both cerebral and peripheral nerve edema (27, 120). AQP-1 is the major isoform in red blood cells, the kidneys, and peripheral nerves (157) 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 colocalizes with AQP, and downregulation of AQP gene transcription and translation. With regard to AQP-4 and to some extent AQP-1, acetazolamide in clinically relevant micromolar concentrations directly blocks water flux across the plasma membrane of oocytes or liposomes in vitro (2, 59, 136), but not all studies confirm these findings in more complex cells (153). Somewhat surprisingly, methazolamide is inactive in blocking water flux (136), despite its very close structural similarity to acetazolamide, differing only by a methyl group substitution on the thia diazole ring (104). 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 coexpression of a catalytically inactive CA mutant that still binds to AQP-1 does not increase water flux (78). How a tight proximity and colocalization 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 water channel of aquaporin.

Acetazolamide also inhibits AQP-1 and AQP-4 gene and protein expression in models of brain and cardiac injury (72, 107), as well as possibly accelerating its proteasomal degradation via ubiquitination (157). It has been reported to reduce vasogenic and cytotoxic forms of cerebral edema in animal models (48, 72). These possible actions of acetazolamide on cerebral edema likely have greater relevance in HACE, but whether subtle changes in brain water in AMS either regionally or below the detection limits of present noninvasive methods are important and pathogenic still remain an unresolved ques-
tion. A recent study in normal-pressure hydrocephalus using MRI scanning found clinical improvement with acetazolamide associated with reductions in interstitial brain water (63); however, to date, no studies of acetazolamide in AMS have examined this possibility. Aquaporin blockade or gene/protein downregulation thus could be salutary in AMS, because slight hyponatremia and hypotonicity (2–3 mM decreases) develop at or before the onset of AMS. This would generate a mechanism whereby, in combination with other factors, extra water could enter brain interstitial and intracellular spaces (12, 43) and aggravate many of the cerebral symptoms of AMS. Studies of aquaporin blockade in AMS prevention would be interesting in this regard.

Interestingly, aquaporins may also serve as channels for small uncharged gas molecules, such as nitric oxide (NO), NH₃, O₂, and CO₂ (55). It is not known whether acetazolamide and other CA inhibitors block aquaporin-mediated CO₂ diffusion across the cell membrane; but, if this is so, this may represent another mechanism by which acetazolamide would cause intracellular CO₂ retention and acidosis to stimulate ventilation, as discussed earlier. The implications, if any, of blockade of AQP-mediated O₂ flux are unknown; but, in animals exposed to 24 h of 10% oxygen, lung AQP-1 is upregulated threefold (1).

**ROS Modulation**

Hypoxic exposure equivalent to typical high altitudes increases ROS formation (10, 83), but studies that have attempted to reduce AMS by administration of varying antioxidant cocktails find opposing results (6, 11). This may be interpreted either as proof of no efficacy or that the particular doses and combinations of antioxidants have not been adequately established. If it is proven that increased ROS formation is responsible for AMS, then acetazolamide, a heterocyclic thiadiazole, might work as an antioxidant, given that other numerous compounds containing a 1-3-4 thiadiazole ring are ROS scavengers (105). Natural defenses against ROS include a number of antioxidant proteins that are upregulated by the gene transcription factor, nuclear related factor-2 (Nrf-2) (86). 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 (86). Whether this difference between the drugs just represents the greater lipophilicity of methazolamide over acetazolamide and great BBB penetration 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 (118, 148).

**Heat Shock Protein and Interleukin-1 Receptor Agonist**

Heat shock protein (HSP)-70 protects against cellular stress induced by hypoxia. In a human study, subjects who did not develop AMS had higher blood levels of HSP-70 than those with AMS, and acetazolamide increased HSP-70 in the AMS-susceptible subjects who did not get AMS (69). Evidence supporting a possible direct salutary effect of HSP-70 upregulation at high altitude was the finding that rats exposed to hypobaric hypoxia and given arimocomol, a HSP inducer, had improved cognitive and motor function compared with control rats (152). In the same human study alluded to earlier (69), acetazolamide also raised the concentration of interleukin-1 receptor agonist (IL-1RA), an anti-inflammatory cytokine. It is not clear whether these findings are due to CA inhibition or to other actions of the drug, such as its acid-base or other off-target effects.

**Hypoxia-Inducible Factor**

The master hypoxic transcription factor, hypoxia-inducible factor 1 (HIF-1), is important in surviving hypoxic stress. When normoxic rats were given very large doses of acetazolamide (50–100 mg/kg), HIF-1α was upregulated in brain tissue (151). Another study of a similar degree of acidosis (pH ~7.0) in cultured cells found moderate upregulation of HIF-1 (149). The relevance of these findings to high altitude is uncertain, because acetazolamide dosing this high causes severe respiratory acidosis by red blood cell CA inhibition. Whether the very slightly lower blood pH with acetazolamide at altitude compared with 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 alters HIF expression, since recent work suggests that the genes for AQP-1 and AQP-4 are HIF responsive and have HIF binding sites (1, 49). For the present, it remains uncertain whether HIF activation underlies AMS or protects against it.

**Other Off-Targets of Acetazolamide**

Recently, it was reported that acetazolamide at a concentration (500 μM) higher than usually attained with administration in AMS (100 μM) increased cAMP in ciliary epithelial cells of the eye (119). The mechanism of action appears to be a stimulation of bicarbonate-sensitive soluble adeny cyclase activity (106), which may be involved in metabolic communication between astrocytes and neurons (28). If this action of acetazolamide applies in the brain where cAMP is a vasodilator and strengthens the BBB (109), then it is possible that acetazolamide may act in this manner to reduce AMS. Acetazolamide at similarly high concentrations also activates a large-capacitance calcium-activated potassium channel (BKCa) in the human vasculature to cause vasodilation (103).

**DEXAMETHASONE AND OTHER CORTICOSTEROIDS**

Corticosteroids, predominantly dexamethasone, have been used for AMS with considerable efficacy since the early 1980s (135). Although these drugs are useful in reducing cerebral edema of many etiologies (72), the mechanism(s) by which they reduce cerebral edema remain enigmatic, particularly at high altitude. Glucocorticoids have many acute (nongenomic) actions that occur rapidly, but also genomic effects taking several hours or more as transcription of many thousands of genes is altered (94). Numerous actions at high altitude of corticosteroids have considerable physiological and clinical rationale: reduction in vascular permeability, suppression of inflammatory pathways, a more favorable antioxidant-oxidant balance, sympatholysis, and improved arterial oxygenation independent of greater ventilation. These protective mechanisms need not be mutually exclusive. Figure 2 provides a brief
Overview of the several potential actions of dexamethasone and other glucocorticoids that are taken up in detail in this section.

Reduction of Vascular Permeability

The BBB is a structure of closely apposed cerebrovascular endothelial cells and a surrounding investment of astrocytes and glial cells that restricts uptake into the brain of most substances in the blood (36). This has the effect of limiting water flux as well as compounds which would be noxious or injurious to neuronal functioning. The potent barrier against paracellular movement of substances into the brain is determined by various proteins that make up the tight junctions between cells, many of which are upregulated with glucocorticoid exposure (36). Hypoxia induces brain capillary endothelial barrier leak (36, 99), and this appears to be dependent upon HIF-1 activity (36). Dexamethasone, while it does not directly suppress HIF-1 expression, appears to block HIF-1 migration to the nucleus and binding to its target genes such as vascular endothelial growth factor (VEGF) (146). VEGF increases brain microvascular permeability (62). Dexamethasone blocks hypoxia-mediated VEGF expression in brain microvascular endothelial cells in culture and can inhibit the accompanying increase in permeability (75). Similar findings of a reduction in vascular permeability as with dexamethasone, along with decreased brain edema in hypoxic rats, with administration of a neutralizing antibody to VEGF have been demonstrated (115). Dexamethasone can also prevent the decline of adenyl cyclase and maintain intracellular cAMP concentrations (109).

Suppression of Inflammation

There has always been a strong current of thought that suppression of inflammatory pathways by glucocorticoids is central to their benefit at high altitude, particularly with decreased expression of nuclear factor kappa beta (NFkB), a transcription factor that initiates production of many proinflammatory genes (30). However, the evidence for hypoxia-mediated inflammation, particularly in humans at altitude (and the accompanying hypoxia), is neither overwhelming nor consistent. In some in vitro studies, the severity of hypoxia needed to generate significant NFkB-driven inflammatory mediator production requires 1–3% O2, but with more realistic altitude-related hypoxia, such as 5% O2, no increases were found for several proinflammatory mediators in alveolar macrophages and endothelial cells (142). With similar hypoxia upward of 24 h, reports vary from no changes in proinflammatory cytokines (67, 133) to some elevation, particularly when exercise and continued residence at high altitude are involved (30, 53). Leukotrienes have been implicated in AMS and intolerance to hypoxia, but there are no changes in circulating inflammatory leukotrienes or improvements in AMS with interruption of leukotriene pathways.

If inflammation is more subtly involved in AMS and not obvious from measurement of circulating cytokines, how then might dexamethasone work that involves inflammatory pathways? What is lacking for obvious practical reasons in humans are tissue (e.g., brain and CSF) measurements which may not
be reflected in serum values of the cytokines mentioned earlier. Other proinflammatory mediators may be relevant. One such cytokine recently examined is monocyte chemotactic protein 1 (MCP-1). It is generated by hypoxic alveolar macrophages and released into the circulation to increase capillary permeability in many systemic beds (26), although the brain was not examined in these studies. Dexamethasone blocks the formation of MCP-1 and a related proinflammatory mediator, macrophage migration inhibitory factor (MIF) (113). In hypoxic rats, dexamethasone via inhibition of MCP-1 blocks inflammatory permeability changes in the microvasculature (25).

HIF-1 appears to activate numerous inflammatory pathways and is also itself upregulated by inflammatory mediators (54). As noted earlier, part of the anti-inflammatory effect of dexamethasone may be to prevent translocation of HIF-1 to the nucleus and thus block HIF-1-mediated proinflammatory gene upregulation (146). HIF-1 also upregulates the glucocorticoid receptor, which will have the effect of moderating HIF-1-mediated proinflammatory cytokine gene transcription and other hypoxia-mediated responses (77). This interplay of HIF-1, glucocorticoids, and glucocorticoids in hypoxia is complicated, and better understanding of their interactions is clearly needed.

Cyclooxygenase-derived eicosanoid inflammatory mediators may be involved in hypoxia-related intolerance and AMS (108), and their production can be inhibited by glucocorticoids (85). While numerous studies have shown that ibuprofen and other nonsteroidal anti-inflammatory drugs reduce the acute headache of high altitude (44), evidence that they reduce AMS is less convincing. The studies are small in number (101), and it is not clear from these studies whether nonsteroidal anti-inflammatory drugs were effective against any of the other symptoms of AMS.

Reduced ROS Formation and Endogenous Antioxidant Upregulation

As discussed earlier, hypoxia and many of its effects may be related to oxidative stress with increased ROS formation. Blood measurements of ROS across the brain in subjects developing AMS reveal a greater generation of oxidized ascorbate and lipid hydroxides and a modest correlation with AMS symptoms, but no evidence of BBB disruption by the appearance of two brain-specific proteins, S110-β and neuron-specific enolase (8, 10). The finding of no escape of large molecular weight brain-specific proteins into the circulation mirrors that found in CSF sampling (9). However, in both cases, the negative results do not rule out lesser BBB disruption that may lead to flux of smaller molecules (<2,000 kDa), such as false neurotransmitter molecules generated in digestion and protein metabolism.

In vitro, dexamethasone inhibits activated microglial cell ROS formation by downregulation of NADPH oxidases (60) and inhibition of mitochondrial complex 1 activity (96). By virtue of its structure, it may itself directly quench ROS, as do other glucocorticoids (70). In mouse models of HACE, dexamethasone reduced brain tissue ROS formation in association with less edema, transvascular leak, and inflammation (115). In part, this protection appears also associated with increases in several antioxidant enzymes and molecules, such as superoxide dismutase, glutathione, and glutathione peroxidase (102), although apparently not by Nrf-2 activation (86). Nonetheless, the use of several antioxidant cocktails in humans for AMS prevention has been disappointing (11, 51). This may reflect a number of factors, including the particular choice of antioxidant compounds and their dosing. A very recent proteomic analysis of persons developing AMS found that they had increases in several antioxidant proteins while those without AMS had no changes (68). These findings suggest two possibilities: either no role of ROS in AMS or, more intriguingly, that quenching ROS activity with high-dose exogenous antioxidants may be counterproductive if endogenous ROS signaling in some manner promotes protection.

Sympatholysis and CNS Actions

Hypoxia activates the sympathetic nervous system, and those with AMS susceptibility have greater sympathetic activation with acute hypoxia (67, 71, 89, 126). This greater activation is even apparent in normoxia, compared with people who are not AMS susceptible (89, 126). Dexamethasone and other glucocorticoids have potent sympatholytic effects in normoxia (114) and at high altitude (92). The beneficial sympatholytic action of glucocorticoids appears linked to suppression of adrenergic tone because propranolol in one small study reduced AMS (42). The immune system and the sympathetic nervous system are intimately interconnected, and increased sympatholytic activity can drive inflammatory events (16) and promote microvascular leakage even independent of inflammation (111). Another possibility is that reduced immune activation itself may limit trigeminal nerve activation (23) and the headache pain of AMS. Given that glucocorticoids can act as anti-emetics and euphoritants, these actions could also account for some of the benefits of dexamethasone in AMS, but there have been no formal studies of other agents with these effects in AMS.

Improved Arterial Oxygenation

CA inhibitors universally improve arterial oxygenation at high altitude (109). Whether dexamethasone does so is not certain. For AMS prevention, a few studies found increased oxygenation at rest (15, 39), augmentation of acetazolamide-induced increase in oxygenation (18), and improved nocturnal sleep oxygenation in HAEPE-susceptible subjects (100). However, in more studies (19, 50, 61, 83, 127) dexamethasone did not increase oxygenation despite impressive prevention and/or moderation of AMS severity. Oxygenation was not greater at maximal exercise in the same group of HAEPE-susceptible subjects noted earlier, when given dexamethasone, but this may have been a result of the considerably higher $V_{O2max}$ attained, in which desaturation worsens with increases in exercise intensity (122). The authors of this study did not report oxygenation values at equal submaximal work rates, which would have been more informative. In the treatment of established AMS, three studies have demonstrated improved oxygenation (38, 50, 93), in which a diuretic effect possibly from a sympatholytic-induced withdrawal of renal sympathetic nerve activity may be postulated.

The mechanism(s) by which dexamethasone could improve arterial oxygenation before AMS onset is not known. Increased ventilation is generally not observed in contrast to acetazol-
amide, since none of the studies of dexamethasone at high altitude or in hypobaric chamber studies cited earlier have measured lower end-tidal or arterial Pco2 values compared with placebo. The only study of dexamethasone and ventilatory responsiveness was done under eucapnic hypoxia and in this somewhat artificial condition without the typical hypocapnia of altitude (129), dexamethasone over a period of 10 h did increase ventilation and the acute hypoxic ventilatory response to a greater degree than placebo (87). The other mechanism by which dexamethasone might improve arterial oxygenation is by enhancement of ventilation-perfusion (V/Q) matching. While no studies with the multiple inert gas elimination technique have been done to assess V/Q matching in a definitive manner, the improvement in the alveolar-arterial PO2 difference with both acetazolamide and dexamethasone that occurs in subjects with AMS is suggestive (93). Improved V/Q matching might be a result of a sympatholytic effect favorably altering neural control of the airways and vasculature of the lungs (130). Other actions of dexamethasone that conceivably might enhance V/Q matching include increased surfactant secretion (103) stimulation of alveolar fluid reabsorption (13) and inhibition of hypoxic pulmonary vasoconstriction (39). More study is clearly needed, particularly to explain why some but not all are better oxygenated when taking dexamethasone.

Other Off-Target Effects of Dexamethasone

As discussed earlier with acetazolamide, alteration in aquaporin expression and water channel activity may also be relevant to dexamethasone in the hypoxic brain. Although it remains uncertain whether the AQP genes have steroid response elements in their promoter regions (141), numerous studies show upregulation of AQP-4 in many forms of edematous brain injury (4) that can be prevented by dexamethasone treatment (32, 33, 47). Edema is moderated in these injuries by other means of AQP-4 suppression, such as AQP-4 knockdown by siRNA (154) and remote ischemic preconditioning (84).

HSP-70 and adrenomedullin are upregulated with dexamethasone in those that are susceptible to AMS (65). As discussed earlier, HSP-70 has considerable pluriotent cytoprotective properties. Adrenomedullin has a BBB-enhancing action (58) as well as having anti-inflammatory (91), diuretic (73), and possibly sympatholytic (73) effects. As a diuretic it may be involved in the finding of possible greater diuresis occurring with treatment with dexamethasone (38).

AMS PATHOGENESIS: IS IT ONLY THE BRAIN?

All previous work has assumed that the action of both drug classes is largely in the CNS by direct engagement with target receptors and modulation of their downstream signaling, with the only exception being the renal action of CA inhibitors to stimulate ventilation by generation of a mild metabolic acidosis. AMS has always been considered to have its origins in the brain and is not thought to affect other organs. Yet numerous studies have shown that the lungs in those with AMS are not entirely spared, as evidenced by slight deterioration in gas exchange, reduction in vital capacity, and lower diffusing capacity compared with those acclimating well (110). When acetazolamide was given for AMS treatment, arterial oxygenation improved more than could be accounted for by the subjects’ increased ventilation (37). Decline in lung function may simply represent changes arising from altered and likely increased efferent activity of the stressed brain in AMS, since vagal innervation of the lung is known to alter airway and pulmonary vascular regulation (69) and possibly cause V/Q mismatching. However, afferent information of inflammatory and other changes in the lung travel to the brain as well, and interruption or alteration of this signaling may be important (94). Aside from the changes in lung function noted earlier, at least in airway and alveolar spaces, there is no evidence of pulmonary inflammation as assessed by bronchoalveolar lavage in those with AMS (97).

Other noninflammatory signaling by the hypoxic lung could be conveyed to the brain via neural pathways. Very recently, it was shown that inhaled budesonide (a high-potency glucocorticoid) was as effective in decreasing AMS as oral dexamethasone (132, 158). Those taking budesonide had slightly better oxygenation and less reduction in vital capacity. Because the pharmacokinetics and dosing of 200-μg budesonide led to blood concentrations well below the dissociation constant for the glucocorticoid receptor but very high concentrations locally in lung tissue (110), its benefit cannot be ascribed to direct steroid receptor engagement in the brain by blood-borne delivery. These findings suggest that hypoxia in the lungs via neural afferent signaling may be involved in initiating critical events in the brain underlying AMS. One source of such afferent information are the neuroepithelial cell bodies (NEB) located in airways which are O2 and CO2 sensitive and are thought to relay information involved in ventilatory control and dyspnea sensation via histamine release (28, 73). Dexamethasone downregulates histidine decarboxylase activity in the lung, although NEB were not specifically studied (131). Last, it is possible that tissue inflammation not appreciable in the air space by lavage (97) is occurring and being suppressed by budesonide. This might fit with MIF and MCP-1 (23, 94) generation in the lungs and suppression locally by air-borne delivery of budesonide. Another possibility for local glucocorticoid action is by stimulation of surfactant secretion (103) with its known anti-inflammatory effects (17). Whether CA inhibitors might act locally in the lung via inhalation for AMS has never been studied, but it is plausible given the evidence that inhaled acetazolamide in humans blocks some forms of irritant-induced cough (32).

CONCLUSIONS

It has been the intent of this review to widen the perspective of how acetazolamide and dexamethasone and analogs of these old hands prevent and treat AMS. What emerges is the strong possibility that both classes of drugs may have pluripotent effects underlying their efficacy, several of which they share in common.

Recognition and investigation of these actions may lead to better understanding of the pathogenesis of AMS, more targeted therapy, and perhaps diminished side effects. This will require structure-function studies of these drugs so that the individual pathways can be isolated by using analogs that are more selective, such as is the case with n-methyl acetazolamide, a non-CA-inhibiting analog. Resolving the extent to which AMS may not solely be a CNS disease, but accompanied or preceded by events in the lung, could be useful in targeting therapy to the lungs by inhalation and avoiding...
systemic side effects. Although no animal models of AMS presently exist with sufficient relevance to a condition that is solely defined by subjective symptomatic criteria, investigations in animals with genetic manipulation of these pathways should be informative, especially if reliable biomarkers or AMS are found.

DISCLOSURES

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