ABSTRACT: Carbonic anhydrase has been localized in skeletal muscle and nerve, thus, inhibition with acetazolamide (ACZ) may alter nerve and/or muscle function in healthy humans. ACZ (3 oral doses 14, 8, and 2 h prior to testing) reduced isometric force (37%) and peak to peak electromyographic (EMG) amplitude (1.38 mV to 0.83 mV), while increasing EMG latency associated with a unilateral Achilles tendon-tap. Reflex recovery profiles, following a contralateral conditioning tap, were similar in both placebo and ACZ experiments. ACZ led to significant changes in H_{max}/M_{max} ratio (52.19/14.42 to 45.73/15.65) and H-reflex latency (34.18 ± 2.54 ms to 35.24 ± 2.74 ms). Motor nerve conduction velocity and maximal voluntary isometric torque (knee extensors) were unaltered by ACZ. These data suggest that inhibition of the tendon-tap reflex and associated isometric force, following ACZ, is related to impairment of synaptic integrity between Ia fibers of the muscle spindle and the alpha motor neuron and not impairment of the muscle spindle or force-generating capacity. © 1997 John Wiley & Sons, Inc. *Muscle Nerve* **20:** 1541–1548, 1997
Key words: afferent transmission; carbonic anhydrase inhibition; H reflex; maximal voluntary contraction; muscle spindle; tendon-tap reflex

ACETAZOLAMIDE REDUCES PERIPHERAL AFFERENT TRANSMISSION IN HUMANS

W.F. BRECHUE, PhD,* D.M. KOCEJA, PhD, and J.M. STAGER, PhD

Human Performance Laboratory, Department of Kinesiology, HPER Building, Room 179, Indiana University, Bloomington, IN 47405, USA

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Carbonic anhydrase (CA) is responsible for catalyzing the reversible conversion between CO_2 and HCO_3. Given its localization to red blood cells, kidney, and lung, CA plays an important role in CO_2 exchange and H^+ regulation. Carbonic anhydrase inhibition (CAI), i.e., following acetazolamide (ACZ), results in CO_2 retention and increased $[H^+]$, and a mild diuresis. ACZ is routinely used in the treatment of glaucoma[23,26] and the prevention and/or amelioration of acute mountain sickness (AMS),[9] as well as for sleep apnea and other respiratory rhythm disorders.[26,37] ACZ efficacy in AMS and the breathing disorders is theoretically related to an increase in ventilation secondary to CO_2 retention and the development of metabolic acidosis.

Our interest in ACZ stems from its use as a prophylaxis for AMS and the implication of CO_2 retention and increased $[H^+]$ upon exercise performance. Intravenous ACZ (1 g 5 min prior to exercise) did not alter power output or fatigue index during short-term bouts of intense exercise, but led to significant reductions in $\dot{V}o_2$, CO_2 output ($\dot{V}co_2$), and significantly increased blood $[H^+]$ following the exercise.[20] When ACZ is administered over a 24-h period (750 mg–1 g), its affects on exercise capacity and tolerance are equivocal. With the chronic dosing paradigm, ACZ has been shown to reduce maximal $\dot{V}co_2$ and time to exhaustion,[21,32,34] with a decrease in sea-level $\dot{V}o_{2max}$,[32] increase in hypoxic $\dot{V}o_{2max}$,[32] or no effect on $\dot{V}o_{2max}$ in either environmental condition.[34] One study has shown no effect of ACZ treatment on $\dot{V}o_{2max}$, $\dot{V}co_{2max}$, or exercise time.[35] These differences in the literature are presently unexplained. However, submaximal exercise time to exhaustion (60–80% of VO_{2max}) is significantly reduced both at sea level and at simulated altitude,[34] with no effect on $\dot{V}o_2$ or $\dot{V}co_2$. In our studies,[2,34] it was concluded that ACZ reduced exercise tolerance due to its diuretic effects and cardiovascular and thermoregulatory dysfunction.[2,34] However, altered H^+ and K^+ homeostasis and its effects on neuromuscular function could not be excluded.[2,34]

CA has been localized in both skeletal muscle[10,18] and nerve tissue.[27–29] Early experiments suggest that

*Correspondence to: Dr. William F. Brechue

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the role of CA in muscle and/or nerve may be regulation of $[H^+]$ via facilitation of CO_2 transport.[13,38] Recent work suggests that skeletal muscle CA may also play a role in excitation–contraction coupling, Ca^{2+} exchange.[7,36] In vitro studies have shown that inhibition of CA leads to reduced isometric force, altered Ca^{2+} transport, and reduced creatine phosphate and adenosine triphosphate.[36] These data suggest that part of the exercise intolerance seen with ACZ in vivo may be related to skeletal muscle dysfunction.

Thus, the purpose of these experiments was to investigate neuromuscular function using the chronic, repeated-treatment paradigm of ACZ in humans, through evaluation of tendon-tap and H reflexes, peripheral motor nerve conduction velocity, and maximal isometric force development.

MATERIALS AND METHODS

Sixteen healthy active males, aged 28.0 ± 4.5 (SD) years, participated in these experiments. All subjects were free of any apparent neurological, orthopedic, or neuromuscular problems. None were on medication prior to or during the study. Each subject received a written and oral explanation of the procedures and was informed of potential risks and benefits in accordance with the University Committee for the Protection of Human Subjects. All subjects gave informed consent of the previously approved protocol prior to commencement of testing.

Experimental Protocol.

Two experimental protocols were employed.

Protocol 1. Each subject (n = 8) participated in two trials (placebo vs. ACZ) to evaluate neuromuscular responses of the Achilles tendon-tap reflex and ulnar nerve conduction velocity.

Protocol 2. Each subject participated (n = 8) in two trials (placebo vs. ACZ) to evaluate the H reflex and maximal isometric knee extension strength. Three subjects participated in both protocols with at least 6 months between protocols.

Treatments.

ACZ was administered in three oral doses of 250 mg 14, 8, and 2 h before testing. To conceal drug identity, flour placebos and ACZ were packaged in unmarked gelatin capsules and administered in identical fashion. All subjects were 10 h postprandial for each experiment with water allowed ad libitum. All experiments were carried out at sea level in a normoxic, thermoneutral environment. Within each protocol, the treatments were administered in a repeated-measures, crossover design for treatment, the order of treatments was randomized, and the drug was dispensed in double-blind fashion. Each trial was separated by 1 week within each protocol.

Tendon-Tap Reflex.

Unilateral and conditioned reflex responses were tested using the Achilles tendon-tap reflex. During reflex testing, the subject sat on a modified Elgin table. Visual input and anticipatory responses were limited by wearing opaque goggles while listening to soothing music through headphones. A description of the apparatus and testing procedures have been previously reported.[15–17] Each foot was rigidly secured to a metal foot plate which prevented movement of the foot/ankle during testing. The knee and ankle were fixed at 90° for all testing. The isometric force output of the reflex response was recorded with a strain gauge that was attached to the metal foot plate. Electromagnetic rubber-tipped solenoids ensured that identical tendon-tap stimuli (equal force applied to the Achilles tendon) were delivered across testing sessions. Tendon-tap force was monitored by placing a piezoelectric force transducer in series with each solenoid. Tendon motion, rather than tap force, determines the stimulus for stretch receptors in the triceps surae such that delivering the same force with identical tendon position and joint angle ensures similar tendon motion (stimuli) across trials.

Bipolar recording electrodes (1 cm diameter) were positioned over the belly of the soleus with a 2-cm intraelectrode distance for examining the Achilles tendon-tap reflex. A third electrode was positioned midway between the two recording electrodes and used as the ground electrode.

Unilateral tendon-tap reflexes were elicited in the right leg without preceding conditioning stimuli. Then, the right leg tendon-tap reflex was elicited following a conditioning tap delivered to the contralateral Achilles tendon to assess the role of crossed spinal input on reflex function. The contralateral conditioning tap preceded the right leg Achilles tendon-tap reflex by 25, 40, 55, 70, 85, 100, 115, 130, and 145 ms. Three trials were administered at each conditioning interval, during each experimental session, for a total of 30 reflex trials per session. Each trial was performed in random order within and across conditioning intervals.

Changes in reflex excitability were determined by examining both electromyographic and force–time characteristics of the reflex response. During each trial, the following dependent measures were recorded: peak isometric force (PF), electromyographic latency (EMGLAT), electromechanical de-

lay (EMD), peak-to-peak EMG, contraction time (CT), and half relaxation time (½-RT).

H Reflex. H reflex was elicited with subjects in the standing position. The stimulating electrodes were placed longitudinally in the popliteal fossa along the tibial nerve. Recording, surface electrodes (Ag-AgCl) were placed over the soleus, approximately 2 cm below the point where the two heads of the gastrocnemius join the Achilles tendon. Electrodes were placed longitudinally on the skin with a 2-cm intraelectrode distance. The ground electrode was placed over the lateral malleolus.

The H reflex and M response were elicited by delivering a percutaneous electrical stimulus (1 ms, square wave pulse) using a square wave stimulator (Grass S88) in series with a stimulus isolation unit (Grass SIU5). The current delivered with each stimulus was monitored with a current probe. On successive trials, the stimulus intensity (current) was increased in 2-mA increments from the first sign of an H wave until a maximal M wave and plateau was observed.

Data Recording. For both tendon-tap and H-reflex testing, the signals from the transducers, the load cell, and the surface electrodes were amplified and interfaced with an IBM computer equipped with an analog/digital conversion board (Data Translations, Model DT-2801). Sampling rate for data collection purposes was set at 2 kHz.

Nerve Conduction Velocity. Ulnar nerve condition velocity was assessed using the double stimulation technique.[33] The ulnar nerve stimulation points were at the elbow in the ulnar notch near the medial epicondyle (S1) and at the wrist just medial to the tendon of the flexor carpi ulnar (S2). Square wave pulses of 0.1-ms duration were applied at each stimulus point with surface-stimulating electrodes. The intensity of the stimulus was adjusted to evoke a supramaximal action potential. All latency measurements were made on a storage oscilloscope (Teca model M). Three latency measurements were made at each stimulation point with the average representing conduction velocity.

The intercathodal distance was measured with an anthropometer. The mean of three distance measurements was used for calculation of motor nerve conduction velocity. Nerve conduction velocity was computed as the distance between proximal and distal stimulation points (S1 and S2) divided by the difference between proximal and distal stimulus latencies.[33]

Maximal Voluntary Isometric Torque (MVIT). Unilateral MVIT of the quadriceps muscle group was measured using a Cybex ergometer. Subjects sat in the machine and the knee joint axis was aligned with that of the machine. Subjects were then strapped and stabilized in that position, with belts across the shoulders, hip/waist area, and the thigh (close to the knee). A pad attached to the movement arm of the machine was strapped to the ankle of the subject. The subject's range of motion was determined with an external goniometer. MVIT was measured at 30°, 45°, 60°, 75°, 90°, and 105° of knee flexion. For each contraction, the joint angle was set, and the machine's movement arm speed was set to 0°/s to allow isometric testing. Subjects were instructed to gradually build up tension against the movement arm by slowly extending the leg (3–4 s duration). Once maximal torque was achieved the subjects were instructed to hold it for 1–2 s, then slowly relax the quadriceps muscle group. Following each isometric contraction the subject received 10–15 s rest while the next joint angle was set.

Statistical Analysis. For analysis of the tendon-tap reflex, a 2 × 10 (condition × interval) analysis of variance (ANOVA) design was used. There were nine conditioning trials, and the nonconditioned trial served as control. Trial by trial intraclass reliability coefficients for the dependent variables in both the placebo and ACZ conditions were between 0.89 and 0.94. Therefore, the average of the subject's three trials at each interval was used as the data point. Analysis of simple main effects was used to examine differences between the two test conditions at each condition interval.

H reflex and torque X angle curves were analyzed by ANOVA with repeated measures, and nerve conduction velocity (NCV) was compared by *t*-test. Post hoc analysis was used where appropriate. The a priori level for all comparisons was set at 0.05.

RESULTS

Unilateral Reflexes. ACZ led to a significant decrease in the isometric force associated with the unilateral tendon-tap reflex (54.6 ± 7.0 N vs. 34.6 ± 6.1 N, Fig. 1C). The decline in isometric force was supported by the EMG activity, which demonstrated a significant reduction (39.9%) in peak-to-peak EMG amplitude following ACZ (1.38 ± 0.14 mV vs. 0.83 ± 0.13 mV, Fig. 1C). There was a significant correlation between force and EMG across trials ($r = 0.86$, $P = 0.02$). ACZ treatment did not alter contraction time following tendon-tap (placebo, PLA, 132.2 ± 8.3 ms

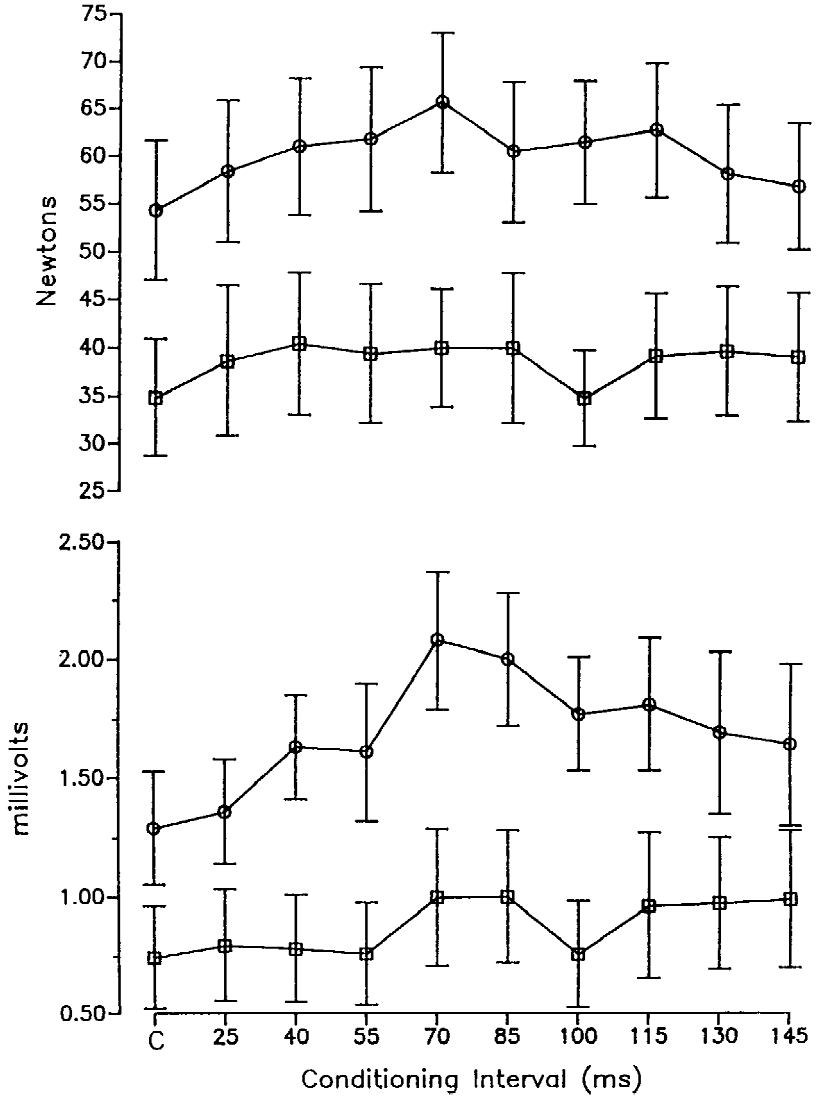

FIGURE 1. Reflex recovery profiles of the Achilles tendon-tap following a conditioning tap applied to the contralateral Achilles tendon. The top curve is peak force, the bottom curve is peak-to-peak EMG. Circles, placebo; squares, ACZ. C is the control point, no conditioning interval. Values are mean ± SD. All ACZ values are significantly reduced from placebo trial, $P < 0.05$.

vs. ACZ, 132.3 ± 9.9 ms). Half-relaxation time of the associated contraction tended to increase (PLA, 84.7 ± 5.3 ms vs. ACZ, 89.3 ± 8.8 ms) following ACZ but was not statistically significant ($P = 0.17$). EMG latency was significantly increased by ACZ treatment from 32.5 ± 1.3 ms to 35.3 ± 1.4 ms with ACZ. Electromechanical delay tended to increase (PLA, 19.5 ± 2.1 ms vs. ACZ, 21.5 ± 1.3 ms) following ACZ but was not statistically different ($P = 0.09$). These data are summarized in Table 1.

Conditioned Reflexes. Assessment of crossed pathways was made by evaluating reflex recovery profiles following a conditioning tendon-tap delivered to the contralateral Achilles tendon. The results of these trials are given in Figure 1. As in the unilateral reflex experiments, ACZ treatment resulted in significantly lower reflex isometric force and decreased peak-to-peak EMG amplitude during the overall conditioning reflex experiments. The reflex recovery profiles following a conditioning tap applied to the contralateral tendon were similar for both groups, the pattern across conditioning intervals being similar with reflex force and EMG observations. Conditioning resulted in slight facilitation in reflex force in both groups.

H Reflex. There was a significant decrease in the H wave (PLA, 5.33 ± 0.11 mV; ACZ, 4.48 ± 0.18 mV) without a change in M wave (PLA, 9.32 ± 0.26 mV;

Table 1. Characteristics of the unilateral tendon-tap reflex, H reflex, and ulnar nerve conduction velocity.

	Placebo	Acetazolamide
Tendon-tap reflex		
Peak force (N)	54.6 ± 7.0	34.6 ± 6.1*
Peak-to-peak EMG latency (mV)	1.3 ± 0.1	0.8 ± 0.1*
EMG latency (ms)	32.5 ± 1.3	35.3 ± 1.4*
Electromechanical delay (ms)	19.5 ± 2.1	21.5 ± 1.3
Contraction time (ms)	132.2 ± 8.3	132.3 ± 9.9
Half-relaxation time (ms)	84.7 ± 5.3	89.3 ± 8.8
H reflex		
H_{max}/M_{max} (%)	52.3 ± 3.2	45.9 ± 3.3*
H-reflex latency (ms)	34.1 ± 0.9	35.3 ± 1.0*
Nerve conduction velocity (m/s)	66.4 ± 2.6	64.1 ± 2.4

Values are mean ± SD, n = 8.
**Statistically different, P < 0.05.*

ACZ, 9.65 ± 0.22 mV). The H_{max}/M_{max} ratio was significantly reduced (PLA, 52.3 ± 3.2%; ACZ, 45.9 ± 3.3%), and the H-reflex latency was significantly increased (PLA, 34.1 ± 0.9 ms; ACZ, 35.3 ± 1.0 ms) following ACZ treatment (Table 1).

Ulnar Nerve Conduction. There were no differences observed in ulnar nerve conduction velocity (Table 1) between placebo (66.4 ± 2.5 m/s) and ACZ (64.1 ± 2.4 m/s).

Maximal Voluntary Isometric Torque. There were no differences in peak torque or torque X angle curves between placebo and ACZ trials (Fig. 2).

DISCUSSION

Our purpose was to evaluate neuromuscular function following treatment with ACZ in intact humans, in vivo, using the tendon-tap reflex, H reflex, ulnar nerve conduction velocity, and maximal voluntary isometric force production. Our data show that ACZ, through CAI, significantly diminishes the Achilles tendon-tap reflex, as indicated by a significant reduction in reflex force generation. Figure 3 is a schematic representation of the monosynaptic stretch reflex evaluated in the present study as the Achilles tendon-tap reflex. There are essentially five components to this reflex: (A) the receptor or muscle spindle; (B) the la fiber; (C) the motoneuron; (D) the motor axon; and (E) the effector or muscle. Our observed reduction in tendon-tap force production following ACZ may be the result of altered nerve function and/or altered muscle contractile function, which could affect any or all of these components (A–D). We will address these components in light of our present data.

Tendon-tap reflex latency was significantly increased following ACZ treatment, suggesting that there is a delay in sensory and/or motor transmission from the initiation of muscle stretch (tap) until the motoneuron delivers impulses to initiate muscle contraction. This impairment could be related to altered function of the muscle spindle receptor (A), 1a afferent nerve conduction (B), motoneuron spinal cord function (C), or alpha motoneuron efferent conduction (D).

The H reflex, as administered here, bypasses the muscle spindle by direct electrical stimulation of the

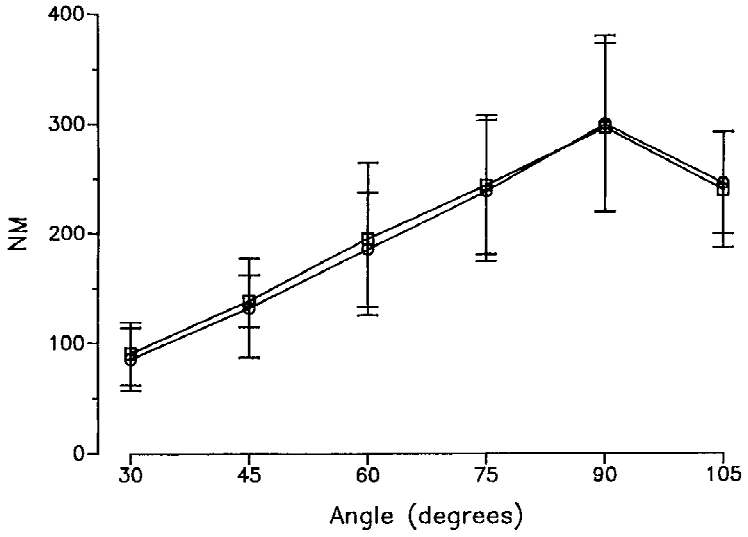

FIGURE 2. Isometric torque X angle curves of the knee extensors. *n* = 8. Circles, placebo; squares, ACZ. There were no significant differences. Values are mean ± SD.

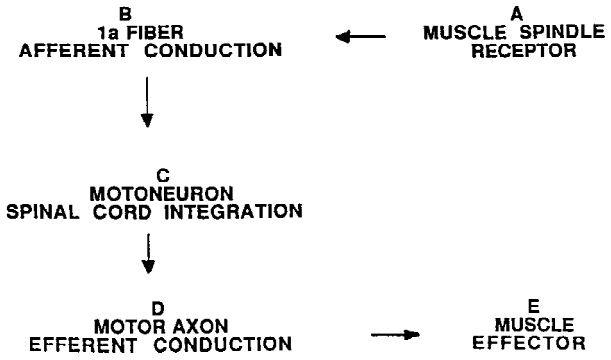

FIGURE 3. Schematic diagram of the tendon-tap reflex.

afferent nerves. Thus, it is possible to discriminate between muscle spindle and afferent nerve function. The H_{max}/M_{max} ratios elicited in the placebo trial (52%) are in agreement with previous work.[31] Given that both the H_{max}/M_{max} ratio and H-reflex latency were significantly reduced following ACZ, it can be concluded that CAI either decreased transmission through 1a afferent neurons and/or impaired the synaptic integrity of 1a afferents and the muscle spindle (Fig. 3B). However, it does not appear that there is impairment of the muscle spindle receptor (Fig. 3A).

Changes in motoneuron excitability and the integrity of crossed-spinal pathways were examined by applying a conditioning tap to the contralateral Achilles tendon and recording the recovery profiles in the ipsilateral stretch reflex. Our data confirm previous research which showed that a conditioning tap to the contralateral Achilles tendon produces motoneuron excitability and facilitation of reflex force in the triceps surae muscle group.[16,17] The new finding is that ACZ treatment did not alter this recovery profile and therefore, had no apparent effect on crossed-spinal integration (Fig. 1). In addition, ACZ had no effect on ulnar nerve conduction velocity, suggesting that motoneuron efferent conduction was unaffected by CAI (Fig. 3D). This establishes that ACZ does not affect efferent function or anything associated with spinal cord function (Fig. 3C) and therefore, is not responsible for the impaired tendon-tap reflex force generation we observed. It is concluded that CAI, with ACZ, produces a deleterious effect on peripheral nerve function that is limited to the la afferent neuron and/or synaptic integrity within the afferent system (Fig. 3B).

Role of Carbonic Anhydrase in Sensory Function. CA has been shown to play an important role in CO_2 sensitivity in both respiratory and lingual chemoreception.[19,24] In both cases it is believed that rapid acidification of intracellular or intraepithelial space, respectively, due to the hydration of CO_2 to H^+ is directly involved in the mechanism of chemoreception.[19,24] CAI has been reported to reduce afferent response to CO_2 in both the phrenic (respiratory center afferent[24]) and trigeminal (lingual afferent[19]) neurons. However, what is not known is whether the reduced CO_2 responsiveness is due entirely to changes in local chemoreception, or if altered afferent transmission is also involved. These previous studies[19,24] recorded from the afferent neuron and could not discriminate between differences in receptor function versus signal conduction. Our present results suggest that afferent transduction might be impaired by altering neuronal signal transduction, given unaltered receptor function in a system (muscle spindle) where the receptor responds to changes in muscle length and is presumably unrelated to CO_2 sensitivity or chemoreception. Thus, it is plausible that impaired respiratory and lingual CO_2 responsiveness may be due in part to altered afferent impulse conduction.

Possible Mechanisms for Impaired Afferent Axon Conduction. The decrease in afferent transmission we observed is likely related to local elevation of CO_2 (P_tCO_2) secondary to CAI. Experimental elevation of P_tCO_2 has been shown to depress spinal mono- and polysynaptic reflexes in animals and humans.[3,14] Elevated P_tCO_2 is reported to hyperpolarize and reduce neuron transmission in nonchemosensitive cells.[8,22] The effect is opposite in chemosensitive cells: neuronal depolarization.[5] The central depressant effect of elevations in CO_2 has been demonstrated in cortical cells, the ambiguus complex, and nonchemosensitive cells in the nucleus tractus solitarii.[5,22] In the peripheral nervous system, somatosensory function is also known to be depressed by elevated CO_2.[22] Further, spinal motoneurons show little, if any sensitivity to elevated CO_2.[22] The authors of one study that showed an apparent CO_2-depressant effect on motoneurons concluded the effect was mediated through inhibition at higher centers,[3] perhaps an effect that includes reduced afferent transmission. The present findings are in agreement with these observations as afferent transmission was reduced without similar effects on motoneuron conduction. These findings are consistent with the idea that elevated CO_2, secondary to CAI, mediates the inhibition of sensory transmission. The sensitivity of afferent neurons, but not efferent neurons to CO_2 is apparently conferred by the selective localization of CA to the afferent side of the central nervous system.[27–29] Thus, the CAI effect is likely

related to elevated CO_2, and depressant effects would be limited to the afferent arm.

Impaired sensory acuity is consistent with commonly reported side effects of ACZ usage. Chronic ACZ therapy has been reported to result in sensory paresthesias, tingling and numbness in the extremities and perioral region,[26] tremors, and tinnitus.[1,23] Likewise, ACZ has been reported to impair the taste of carbonated beverages, i.e., they taste noxious and flat.[25] Ataxia[1,23] and premature muscle fatigue[34] are also noted following ACZ treatment. The muscle spindle and golgi tendon organ work in concert to provide local feedback regarding muscle length and tension. With impaired afferent transmission, the central nervous system is likely to receive inappropriate signals from skeletal muscle concerning locomotion. This could result in inappropriate motor unit recruitment (as seen in the reflex experiments) and altered/reduced skeletal muscle activity. Further investigation of the contribution of altered sensory transmission to impaired locomotor function or skeletal muscle fatigue is needed.

Skeletal Muscle Function. Recent evidence,[7,36] suggests that CA plays an important role in skeletal muscle contractility and thus, direct effects of CA inhibition on skeletal muscle contraction and the decrease in reflex isometric force are plausible.

CA has been localized to the sarcoplasmic reticulum,[4] cytosol, and sarcolemma in skeletal muscle.[7,36] Isometric force development has been shown to be impaired following CA inhibition in vitro, apparently related to increases in intracellular inorganic phosphate and/or decreased $[H^+]$.[7,30,36] CA inhibition in skeletal muscle has been shown to alter Ca^{2+} kinetics, which leads to increased time to peak of twitch contractions in vitro, and delayed relaxation time, independent of $[H^+]$.[36] In addition, ACZ, as administered here, results in a slight metabolic acidosis.[2,32,34,35] Increased $[H^+]$ has been shown to reduce the force-generating capacity of skeletal muscle, in vitro.[6]

In the present study, ACZ led to a decrease in the reflex isometric force response to a tendon-tap, that was associated with a concomitant reduction in EMG activity. The significant correlation between force generation and EMG activity ($r = 0.86$) across trials suggests that changes in force were closely related to changes in muscle activation. The reduction in electrically induced H wave, with the same stimulus, further supports decreased motor unit recruitment following ACZ. Thus, it would appear that the decrease in tendon-tap force was related to reduced motor unit recruitment rather than impaired muscle contractile function directly related to CAI[7,36] or secondary to acidosis.[6] No differences in maximal voluntary force production (torque X angle) were observed between groups, further evidence that the contractile apparatus is intact following ACZ. Thus, there appears to be no direct effects of CAI (elevated CO_2 or H^+) on skeletal muscle force, confirming several studies that have shown that metabolic acidosis does not affect voluntary or involuntary force production in humans.[11,12] The lack of a specific effect of CA inhibition or metabolic acidosis on force production in vivo, as seen in vitro, remains to be explained.

In conclusion, commonly used therapeutic doses of ACZ significantly alter in vivo tendon-tap reflex and reflex-associated isometric force in intact man. This reduction appears to be related to impaired la afferent nerve transmission and/or impaired synaptic integrity. In the present context, the effects of ACZ on nerve function appear to be limited to the periphery, since crossed-spinal pathways were not affected, and to the afferent arm, as motoneuron conduction velocity was not changed. The reduction in reflex force production appears to be related to altered motor unit recruitment secondary to impaired sensory transmission, rather than CA-dependent or $[H^+]$-dependent effects on muscle contractile function or contractility.

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