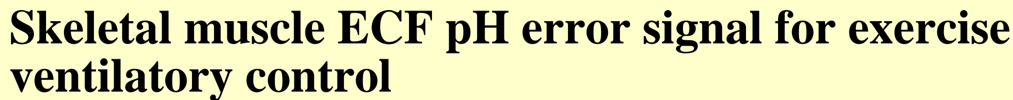

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Skeletal muscle ECF pH error signal for exercise ventilatory control

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Evans, Allison B., Larry W. Tsai, David A. Oelberg, Homayoun Kazemi, and David M. Systrom. Skeletal muscle ECF pH error signal for exercise ventilatory control. *J. Appl. Physiol.* 84(1): 90–96, 1998.—An autonomic reflex linking exercising skeletal muscle metabolism to central ventilatory control is thought to be mediated by neural afferents having free endings that terminate in the interstitial fluid of muscle. To determine whether changes in muscle extracellular fluid pH (pH_e) can provide an error signal for exercise ventilatory control, pH_e was measured during electrically induced contraction by ^{31}P-magnetic resonance spectroscopy and the chemical shift of a phosphorylated, pH-sensitive marker that distributes to the extracellular fluid (phenylphosphonic acid). Seven lightly anesthetized rats underwent unilateral continuous 5-Hz sciatic nerve stimulation in an 8.45-T nuclear magnetic resonance magnet, which resulted in a mixed lactic acidosis and respiratory alkalosis, with no net change in arterial pH. Skeletal muscle intracellular pH fell from 7.30 ± 0.03 units at rest to 6.72 ± 0.05 units at 2.4 min of stimulation and then rose to 7.05 ± 0.01 units ($P < 0.05$), despite ongoing stimulation and muscle contraction. Despite arterial hypocapnia, pH_e showed an immediate drop from its resting baseline of 7.40 ± 0.01 to 7.16 ± 0.04 units ($P < 0.05$) and remained acidic throughout the stimulation protocol. During the on- and off-transients for 5-Hz stimulation, changes in the pH gradient between intracellular and extracellular compartments suggested time-dependent recruitment of sarcolemmal ion-transport mechanisms. pH_e of exercising skeletal muscle meets temporal and qualitative criteria necessary for a ventilatory metaboreflex mediator in a setting where arterial pH does not.

acid-base; magnetic resonance spectroscopy; metaboreflex; ventilation; extracellular fluid

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THE EXQUISITE MATCHING of ventilation and blood flow to the demands of exercising skeletal muscle has long been recognized. Despite nearly a century of work, however, the autonomic reflexes responsible for oxygenation and acid-base homeostasis when the metabolic rate is elevated remain poorly understood (6). One such putative pathway is the muscle "metaboreflex" whereby group IV unmyelinated nerves, stimulated by certain by-products of metabolism (9, 14, 18, 24), communicate with regions of the central nervous system (CNS) important in the regulation of cardiorespiratory function. Evidence for a muscle chemoreflex is strongest in the case of the systemic pressor response (10, 14, 18, 25), but a growing body of literature suggests it may be important for ventilatory control as well. The latter has received support from a series of elegant neurophysiological experiments in cat (1, 14, 23, 24) and from recent clinical studies that suggest the pathway is operative in the normal (4, 36) human and in disease (21).

Candidate metabolites relevant to the afferent limb of the reflex have been proposed on the basis of their increased concentration in exercising muscle (24) or their venous (23) blood and associated neural responses (14, 23, 24). Although lactic acid seems to lead the list of likely candidates (24), dynamic measurement of lactic acid and pH in the extracellular fluid (ECF) of exercising muscle has proved difficult. Because the extracellular space constitutes only approximately one-fourth of skeletal muscle volume (19), a whole muscle biopsy cannot yield useful information about metabolite concentrations in the microenvironment of free nerve endings. Glass pH electrodes with diameters ranging from 900 (24) to 50 µm (30) have been used to estimate pH in the ~0.5-µm interstitial space of skeletal muscle, but they introduce the possibility of tissue damage and contamination by blood and intracellular fluid.

^{31}P-magnetic resonance spectroscopy (MRS) allows the noninvasive measurement of intracellular pH (pH_i) because nearly all of the phosphorus in solution is intracellular and because diprotonation of P_i causes a change in its nuclear magnetic resonance (NMR) frequency (20). The addition of a phosphorylated marker that distributes exclusively to the extracellular space (7, 19) should allow nondestructive measurement of extracellular pH (pH_e) if its negative logarithm of the acidic dissociation constant (pK_a) is in the physiological pH range and if it possesses a distinct ^{31}P-MRS-visible peak, with a resonance that is pH sensitive. Such a marker exists [phenylphosphonic acid (PPA)] that is nontoxic and has a pK_a of 7.01 (7, 19).

In the present study, PPA was used to measure muscle pH_e in the anesthetized rat while the sciatic nerve was electrically stimulated. The study was designed to determine whether skeletal muscle pH_e, measured nondestructively by ^{31}P-MRS, could function as an error signal mediating the metaboreflex. We hypothesized that, if extracellular hydrogen ion is important, it must change concentration rapidly in ECF, in a direction known to activate the ventilatory metaboreflex, and must do so when arterial pH (pH_a) is thought to play little or no role in augmenting ventilation.

METHODS

Animal Preparation

Seven male Sprague-Dawley rats (205–295 g) were sedated with intramuscular midazolam (0.1 mg/kg). Light anesthesia was induced with ketamine (75 mg/kg im) and

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maintained with ketamine infused at 100 $mg \cdot kg^{-1} \cdot h^{-1}$ through an intraperitoneal catheter while the animal was allowed to breathe spontaneously. Silastic tubing (0.5 mm ID) was placed in the right internal jugular vein. Vascular volume expansion was accomplished by the infusion of 6% hetastarch (15 ml/kg) for 45 min followed by 0.9% saline (10 ml/kg) through the internal jugular vein over 1 h. Expansion was necessary to prevent hypotension in the upright quadruped. Silastic tubing was also placed in the left carotid artery and connected to a pressure transducer (model 1290C, Hewlett-Packard, Waltham, MA) and strip-chart recorder (model 7754B, Hewlett-Packard) for measurement of mean arterial blood pressure and for blood sampling. The right hindlimb was shaved, and nonmagnetic platinum electrodes were attached to the right sciatic nerve with Parafilm (American National Can, Greenwich, CT) insulating the connection. The sciatic nerve proximal to the electrode attachment was sectioned to reduce retrograde transmission during stimulation.

Skeletal Muscle pH_e Measurement

PPA (mol wt 158.09) was obtained from Aldrich Chemical (Metuchen, NJ). A 675 mM solution of PPA was prepared by adding distilled water to the powder, and sodium hydroxide was added until a pH of 7.40 was attained. Cold sterilization was performed by using a Millex-GS 0.22-μm filter (Millipore, Bedford, MA). PPA distributes to the extracellular space and provides an accurate measure of pH_e by ^{31}P-MRS without cytotoxicity or metabolic effects (7, 19). A 3-ml PPA loading dose was infused through the internal jugular line over the 15 min before scanning, followed by a continuous infusion at 2 ml/h. Core temperature of the animal was maintained at 36–37°C by adjusting an external heat source.

^{31}P-MRS

Animals were placed in an aluminum probe with the right gastrocnemius muscle secured over a 1.4-cm-diameter surface coil. The probe was then inserted into a vertical 8.45-T NMR magnet, interfaced with a Nicolet NT 360 spectrometer (Nicolet Instruments, Madison WI). The homogeneity of the magnetic field was optimized by shimming on the proton signal of muscle water. The coil was then tuned to 145.75 MHz for phosphorus. Sweep width was 6,000 Hz. Spectra were acquired from 20-μs radio-frequency pulses with a 3-s repetition time. A resting spectrum was obtained by using an average of 100 free induction decays (FIDs). During electrically induced contraction and recovery, 24 FIDs were averaged per spectrum.

Rest, Stimulation, and Recovery

Exercise was simulated by continuous 5-Hz sciatic nerve stimulation for 15.6 min, followed by a 14.4-min recovery period. Stimulator voltage was set at twice that required to elicit maximal gastrocnemius contraction at 5 Hz and was not varied throughout the protocol. Pilot studies outside of the magnet confirmed that muscle continued to contract throughout the stimulation protocol. Systemic blood pressure was monitored continuously, and a 200-μl aliquot of arterial blood was withdrawn at rest, every 1.2 min during electrically induced contraction, and in duplicate at the end of recovery. Arterial blood was analyzed for P_{O_2}, P_{CO_2}, and pH at 37°C (Ciba-Corning Diagnositics, Medfield, MA) and for lactate concentration (Analox Instruments, London, UK). HCO_3^- concentration was calculated from the Henderson-Hasselbalch equation.

Data Analysis and Statistics

Spectral analysis was performed by using a Sun 3/260 workstation. FIDs were zero filled to 4K and multiplied by an exponential corresponding to a 20-Hz line broadening before fast Fourier transformation, followed by phasing, baseline correction, and Lorentzian curve fitting.

pH_i was determined by measuring the chemical shift of the P_i peak relative to phosphocreatine (PCr) according to the equation

$$pH_i = 3.07124 + \delta(0.8291)$$

where δ is the difference in resonance frequency between PCr and P_i peaks in parts per million. pH_e was determined by using the chemical shift between PPA and PCr peaks by the equation

$$pH_e = (23.6007 - \partial)/1.1755$$

where ∂ is the difference in resonance frequency between PPA and PCr peaks in parts per million.

PCr and P_i curves were integrated, and the ratio of PCr area to that of P_i was used as an estimate of phosphorylation potential of skeletal muscle mitochondria.

Unless otherwise stated, data are expressed as mean ± SE of data collected during the preceding 1.2-min period. Comparisons among resting, 5-Hz stimulation, and recovery data were made by using the paired *t*-test, analysis of variance for repeated measures with a Fisher's post hoc test, and 95% confidence intervals for linear regression slope and intercept. Values were considered to be in a steady state when the 95% confidence intervals for slope overlapped zero. Statistics were performed by using Statview software (Abacus Concepts, Berkeley, CA). $P \leq 0.05$ was considered significant.

RESULTS

Stability of the Model

At the resting baseline, arterial blood revealed a mild mixed metabolic (lactic) acidosis and respiratory alkalosis (Table 1). At the end of recovery (Table 1), blood pressure and arterial oxygenation were normal. Frequent blood sampling did not result in excessive anemia (end-recovery hemoglobin = 12.5 ± 0.6 g/dl). Arterial P_{CO_2}, HCO_3^-, and pH returned to baseline, but blood lactate and pH_i had not fully recovered. The intracellular PCr-to-P_i; ratio (PC_r/P_i) returned to resting baseline values (Fig. 1).

Table 1. *Metabolic and spectroscopic results at rest and at end of recovery*

	Rest	End of Recovery
BP, mmHg	111 ± 2	100 ± 5
Pa_{O_2}, Torr	95 ± 5	112 ± 4*
Pa_{CO_2}, Torr	34 ± 1	31 ± 1
Lactate, mM	1.82 ± 0.20	4.03 ± 0.69*
HCO_3^-, mM	22.5 ± 1.0	21.6 ± 1.5
pH_a, units	7.41 ± 0.01	7.42 ± 0.02
pH_i, units	7.30 ± 0.03	7.13 ± 0.07*
pH_e, units	7.40 ± 0.01	7.35 ± 0.03

Values are means ± SE. Pa_{CO_2}, arterial P_{CO_2}; Pa_{O_2}, arterial P_{O_2}; pH_a, arterial pH; pH_i, intracellular pH; pH_e, extracellular pH. * $P < 0.05$, end of recovery vs. rest.

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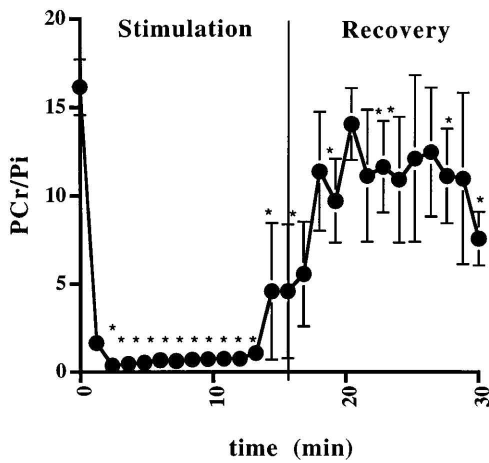

Fig. 1. Phosphocreatine (PCr)-to-P_i ratio during rest (*time 0*), stimulation, and recovery. Values are means ± SE. Fall from and subsequent return toward baseline PCr/P_i values indicate contraction during stimulation and confirm the viability of the preparation. * $P <$ 0.05 vs. baseline.

Acid-Base Regulation at Rest and During Stimulation

Arterial blood. Sciatic nerve stimulation exaggerated both the resting lactic acidemia and the superimposed respiratory alkalemia (Fig. 2). Lactate concentration increased from 1.8 ± 0.2 mM at rest to a peak of 6.9 ± 1.2 mM at 4.8 min of stimulation ($P < 0.05$) and then remained stable throughout stimulation. pH_a showed no net change vs. resting baseline ($P > 0.05$; Fig. 3).

Intracellular space. pH_i fell from a resting value of 7.30 ± 0.03 to a nadir of 6.72 ± 0.05 units ($P < 0.05$) after 2.4 min of stimulation, partially recovered, and then achieved a new mean steady-state value of 7.05 ± 0.01 units from time (t) = 6–15.6 min (Fig. 3), which was more acidic than that at rest ($P < 0.05$).

The initial ($t = 0$–2.4 min) pH_i fall was linear vs. time ($y = 7.29 - 0.24x$; $P < 0.05$, $r = 0.93$). The subsequent ($t = 2.4$–6.0 min) partial pH_i recovery was also linear vs. time ($y = 6.50 + 0.09x$; $P < 0.05$, $r = 0.65$).

Extracellular space. Resting pH_e (7.40 ± 0.01 units) was not different from that of the arterial compartment (7.41 ± 0.01 units; $P > 0.05$) but was more alkalotic than resting pH_i ($P < 0.05$). With stimulation, the pattern of pH_e change was an attenuated and slightly delayed version of that in the intracellular compartment (Fig. 3). The maximum decrease in pH_e (7.16 ± 0.04 units) occurred 1.2 min after the nadir of pH_i (Fig. 3).

The initial ($t = 0$–2.4 min) pH_e fall was linear vs. time ($y = 7.39 - 0.09x$; $P = 0.05$, $r = 0.69$) but fell less steeply than did pH_i (95% confidence intervals for slope = −0.14 to −0.05 vs. −0.29 to −0.20 unit/min, pH_e vs. pH_i). Whereas pH_i partially recovered (t = 2.4–6 min), pH_e remained stable at a pH of 7.18 ± 0.02 units, which was acidic vs. its resting baseline ($P <$ 0.05). pH_e remained alkalotic (7.22 ± 0.01 units) relative to pH_i ($P < 0.05$) for the remainder of electrically induced contraction (t = 6.0–15.6 min).

The pH gradient between extracellular and intracellular spaces peaked at 0.47 ± 0.06 unit at t = 2.4 min of electrically induced contraction (Fig. 4) and then fell to a steady-state value of 0.18 ± 0.02 unit for t = 4.8–14.4 min.

Recovery

Arterial blood. Arterial P_{CO_2} and HCO_3^- returned to their respective resting baselines by the end of recovery ($P > 0.05$ for each; Table 1, Fig. 2), with no net change for pH_a (Table 1, Fig. 2). Arterial lactate decreased only to 4.0 ± 0.7 mM, which remained different from the value at rest ($P < 0.05$; Table 1, Fig. 2).

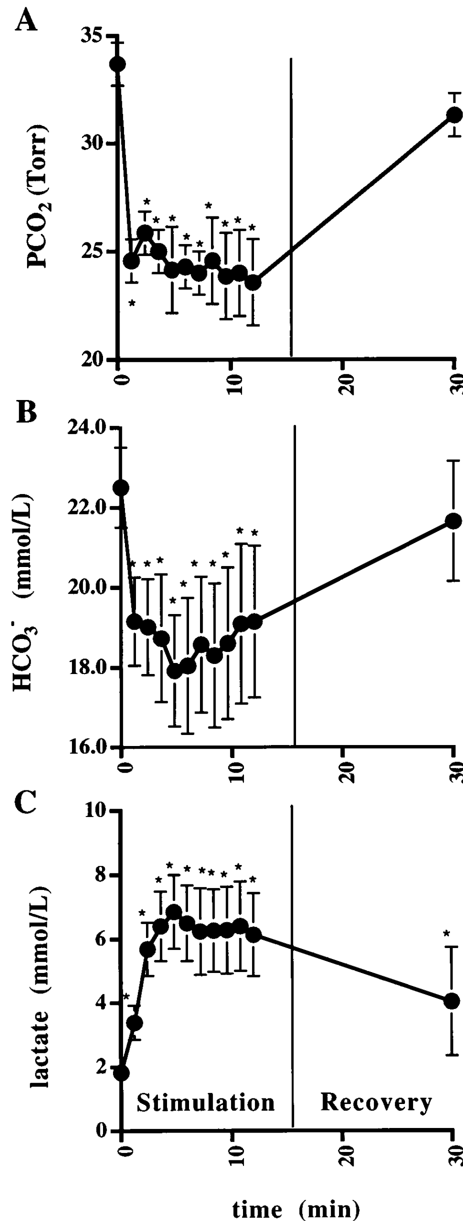

Fig. 2. Arterial blood P_{CO_2} (*A*), HCO_3^- (*B*), and lactate concentrations (*C*) during rest (*time 0*), stimulation, and recovery. Values are means ± SE. Arterial P_{CO_2} and HCO_3^- fully recovered, whereas lactate did not. * $P < 0.05$ vs. baseline.

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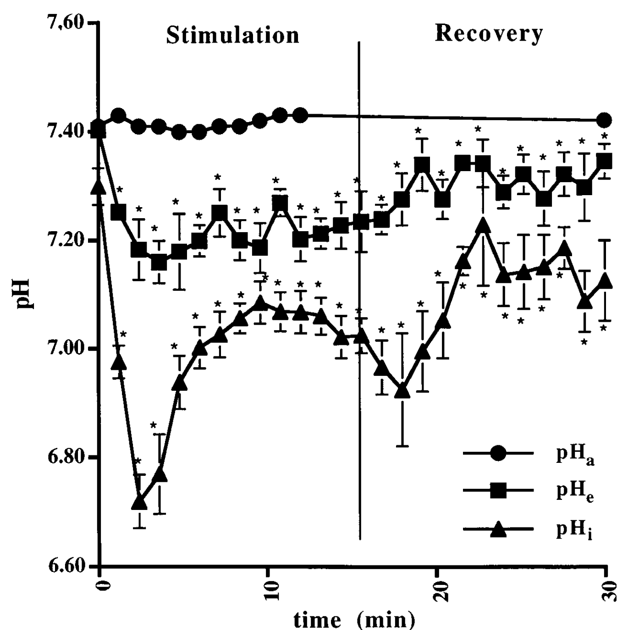

Fig. 3. Arterial pH (pH_a), skeletal muscle extracellular pH (pH_e), and intracellular pH (pH_i) during rest (*time 0*), stimulation, and recovery. Values are means ± SE. Despite hypocapnia and arterial isohydria, pH_e falls rapidly and in sustained fashion to acid pH and could thus function as error signal for ventilatory metaboreflex. * $P < 0.05$ vs. baseline.

Intracellular space. On cessation of stimulation, pH_i remained unchanged for 2.4 min ($P > 0.05$; $t = 2.4$ min of recovery vs. last stimulation point) but then began a rapid (95% confidence intervals for slope = 0.02–0.11 unit/min) linear ($y = 5.76 + 0.06x$; $P < 0.05$, $r = 0.47$) rise over the ensuing 4.8 min to a new steady-state value of 7.15 ± 0.02 ($t = 6$–14.4 min of recovery).

Extracellular space. As opposed to pH_i, pH_e began to recovery immediately but more gradually (confidence intervals for slope = 0.01–0.06 unit/min). The new steady-state value of 7.32 ± 0.01 units ($t = 6$–14.4 min of recovery) was more alkaline ($P < 0.05$) than that of the intracellular space.

In a fashion analogous to that during stimulation, the pH gradient between intra- and extracellular spaces peaked at 0.35 ± 0.08 unit at $t = 2.4$ min of recovery and then fell to a steady-state value of 0.17 ± 0.02 unit for the remainder of recovery ($t = 4.8$–14.4 min; Fig. 4). Peak pH gradients were not different during recovery vs. contraction ($P > 0.05$). Similarly, steady-state pH gradients were not different when recovery was compared with electrically induced contraction ($P > 0.05$). The relationship between pH_e and pH_i during rest, stimulation, and recovery was best described (least residual sum of the squares) by a second-order polynomial $y = 34.94 - 8.27x + 0.62x^2$ ($r = 0.76$, $P < 0.05$; Fig. 5).

A stepwise multiple regression included pH_i as an independent variable for pH_e, but it excluded pH_a. This was true during both stimulation and recovery and when data from the two periods were pooled.

DISCUSSION

The skeletal muscle metaboreflex, mediated by unmyelinated group IV afferent nerves, which are stimulated by metabolites in the ECF of exercising muscle (9, 14, 18, 24) and processed by glutamatergic receptors in the lumbosacral spinal cord (1), may be important in ventilatory control (2, 4, 11, 13, 14, 18, 21, 23, 24, 34, 36). Candidate metabolites have been proposed on the basis of their increased concentration in exercising muscle (24) and blood (23) and associated neural responses (14, 23, 24). Although lactic acid has emerged as a major contender (24), measurement of its concentration or associated pH change in the ECF of exercising muscle has been difficult (24, 30). ^{31}P-MRS, in conjunction with a phosphorylated marker confined to ECF, allows noninvasive measurement of exercising skeletal muscle pH_e. We hypothesized that pH_e would become rapidly acidic in a setting where arterial P_{CO_2}

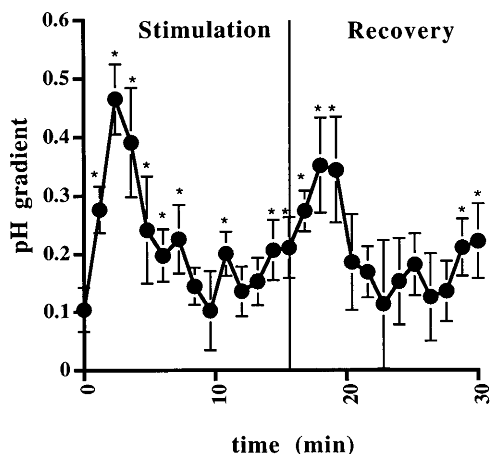

Fig. 4. Skeletal muscle pH gradient between extracellular and intracellular compartments. Values are are means ± SE. Maximum gradient occurred during on- and off-transients of stimulation. * $P < 0.05$ vs. baseline.

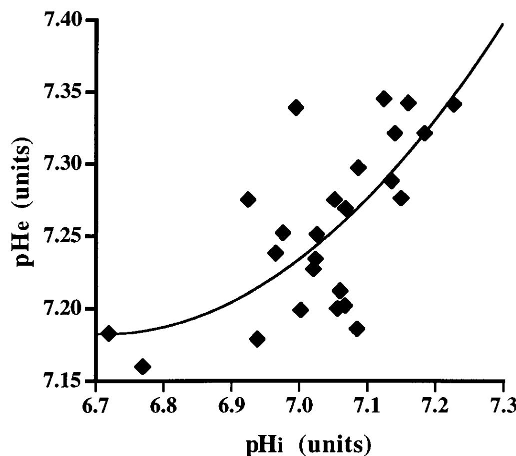

Fig. 5. Skeletal muscle pH_e vs. pH_i at rest, during contraction, and at end of recovery. Relationship is best described by the second-order polynomial $y = 34.94 - 8.27x + 0.62x^2$ ($r = 0.76$, $P < 0.05$) and suggests recruitment of a saturable, pH_i-sensitive sarcolemmal ion-transport system.

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and pH should not stimulate ventilation (6). The major finding was that pH_e does, in fact, meet all of these criteria and thus could provide an error signal for exercise ventilatory control.

The Model

Continuous, unilateral 5-Hz sciatic nerve electrical stimulation results in a stable model that mimics most of the arterial and skeletal muscle acid-base changes found in short-term human volitional exercise. Despite unilateral stimulation and the recruitment of a muscle mass that is small relative to the volume of distribution for by-products of metabolism, arterial lactate rose to levels found during incremental exercise in humans and HCO_3^- changed in a reciprocal fashion. The pattern of the PCr/P_i ratio fall was also similar to that seen during incremental exercise in humans (32); its low values throughout the bulk of the protocol suggest that transmission fatigue (3) did not occur and that muscle continued to contract. pH_i also fell to values similar to those reported during brief, intense exercise in humans (32).

A feature of the model different from human volitional exercise was a superimposed primary respiratory alkalemia, despite sectioning of the proximal sciatic nerve and the use of insulating material at the site of electrode attachment. Some, but not all, of the peripheral stimulus for hyperventilation could have come from the rapidly rising blood lactate concentration during contraction and resulting arterial chemoreceptor activity. Another possibility is retrograde propagation of current via tissue and spinal cord in series (5). A final, but unproved, hypothesis is that an intact metaboreflex was mediated via intact sympathetic afferents associated with blood vessels. Irrespective of the mechanism, the model provided an opportunity to examine the afferent limb of the skeletal muscle metaboreflex under hypocapnic conditions, which minimize afferent input from classic chemoreceptors (carotid bodies and CNS) (6, 29).

Intracellular Space

pH_i fell quickly during contraction and partially recovered as stimulation continued. Takata and colleagues (33) found a similar pattern when they stimulated rat hindlimb for 20 min at two separate frequencies.

The Stewart analysis (31) of acid-base control states that hydrogen ion concentration of a physiological solution is dependent on the strong ion difference (SID), P_{CO_2}, and the total concentration and dissociation constant of weak acids and bases ($[A_{tot}]$). During brief, intense exercise similar to that elicited in the present study, two-thirds of the rise in exercising skeletal muscle intracelluar hydrogen ion concentration is mediated by narrowing of the SID, which is in turn due to accumulation of lactate and efflux of K^+ from the cell (17). Another 25% of the increased intracellular hydrogen ion concentration is related to increased total concentration of weak acids such as the histidine groups of protein and P_i as well as their collective apparent equilibrium constant. Only 10% of the increase in intracellular hydrogen ion is thought to be dependent on P_{CO_2}.

Several mechanisms could explain partial recovery of pH_i during stimulation, including decreasing skeletal muscle contraction and/or glycolytic flux, increased buffering capacity, and activation of sarcolemmal ion-transport mechanisms. Impairment of excitation-contraction coupling has been described during stimulated contractions (3), and, over time, attenuation of lactate production could have ensued. The PCr/P_i ratio, however, a surrogate marker of cellular metabolic stress, showed a sustained decrease throughout stimulation, suggesting ongoing contraction. Second, glycolytic flux and ATP production might have been decreased due to hydrogen ion inhibition of muscle phosphorylase or phosphofructokinase activity. In the present study, however, any tendency toward enzyme inhibition by intracellular acidosis should have been more than offset by activation through increased AMP and IMP and the observed rise in P_i (8). Third, PCr^{2-} hydrolysis consumes a proton, results in loss of a strong anion, and increases buffering capacity through accumulation of P_i. These changes could have progressively mitigated the pH_i fall in the face of ongoing proton production. This is unlikely, however, because PCr/P_i ratio changes were complete well before pH_i began to recover.

By default, therefore, the most reasonable explanation for partial pH_i recovery may be a time- and pH_i-dependent (15) recruitment of active sarcolemmal carrier mechanisms for lactate (22, 26, 35). This is supported by examination of the temporal pattern of change of the pH_i-pH_e gradient, which was large only during the on- and off-transients of electrically induced contraction. In addition, modeling of the pH relationship between intra- and extracellular compartments (Fig. 5) suggests that, although such transmembrane transport systems may be pH_i dependent (15), they are also saturable (22).

Extracellular Space

pH_e did in fact change promptly in an acid direction at the onset of stimulation and remained so throughout electrically induced contraction, under hypocapnic conditions known to depress carotid body (29) and CNS (12) chemoreception. Skeletal muscle pH_e, therefore, meets the criteria necessary for an error signal mediating the ventilatory metaboreflex (23, 24).

Despite its potential importance, acid-base regulation in the extracellular space of exercising muscle has received little attention in the literature. Rotto and colleagues (24) found a normocapnic (arterial P_{CO_2} = 31 Torr, pH_a = 7.39 units) resting "intramuscular" pH in cat of 7.28 units, which fell to 7.13 units as a result of static muscle contraction. They recognized, however, that their use of a 900-μm electrode likely caused tissue damage and could only estimate pH_e (24).

Steinhagen et al. (30) used electrodes with diameters that ranged from 50 to 100 μm and reported a resting

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normocapnic pH_e of 7.27 units. With sciatic nerve stimulation and end-tidal P_{CO_2} maintained at 40 Torr, pH_e became alkaline for 15 s, fell over 3 min to a nadir of 6.98 units, and then partially recovered over 12 min to a pH_e of 7.11 units, during ongoing stimulation. These values are very similar to those found in the present study. Some of the differences might be explained by time resolution of pH electrode vs. MRS and by the relative hypercapnia in the earlier study. Histological study of the lesions caused by the needle electrode, however, showed evidence of tissue damage, introducing the possibility that the ECF was not exclusively sampled. Meyer and colleagues (19) perfused resting cat muscle with a solution containing 5% CO_2 and found a pH_e of 7.18 units by the chemical shift of PPA in the ^{31}P-MRS. These investigators suggest, as do we, that pH_e of skeletal muscle is regulated at a value between that of the intracellular space and blood.

Consideration of the physicochemical determinants of pH_e leads to some interesting speculation. As opposed to the intracellular compartment of muscle and plasma, the concentration of protein buffers in the extracellular compartment is small (30). In addition, contributors to intracellular $[A_{tot}]$ such as P_i and ATP (17) are effectively excluded from the interstitial space. Finally, the water content of skeletal muscle ECF doubles during intense exercise (28), decreasing $[A_{tot}]$ even further. Thus, in the ECF of muscle, buffering capacity is low and pH_e will be disproportionately determined by SID and P_{CO_2}.

The SID of skeletal muscle ECF is largely determined by the relative concentrations of lactate and K^+. During exercise, K^+ efflux during repolarization exceeds influx via the Na^+-K^+ pump. Calculated extracellular K^+ concentration increases by only 2 mM (27), which would increase SID by the same amount. Extracellular lactate concentration, on the other hand, increases by nearly 10 mM (27); therefore, SID would be expected to narrow by ~8 mM and acidify the ECF of muscle. With intense exercise, the P_{CO_2} of venous blood draining muscle exceeds 100 Torr (16). As opposed to the small influence of P_{CO_2} on pH_i (16, 17), P_{CO_2} is responsible for most of the acid change in venous blood (16). This should be even more pronounced in the ECF of skeletal muscle, which would be exposed to slightly higher P_{CO_2} than would plasma and which has a buffering capacity ($[A_{tot}]$) that is even less (30).

We conclude, by using noninvasive MRS techniques, that a pH error signal for the metaboreflex does exist in the ECF of exercising skeletal muscle, even in the presence of arterial hypocapnia. We speculate that the interstitial space of muscle is an ideal location for free nerve endings involved in cardiorespiratory control. The lack of protein buffer in the extracellular space could provide "gain" to such a reflex, making it exceedingly responsive to by-products of increased muscle activity such as CO_2 and lactate anion. Because the system is especially influenced by P_{CO_2}, it could play a major role in ventilatory control throughout incremental exercise, including the submaximal "isocapnic" phase.

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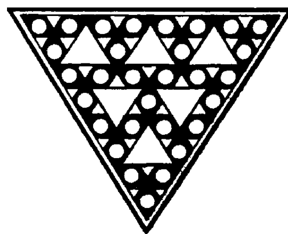

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