Reflex cardiovascular and ventilatory responses to increasing H^+ activity in cat hindlimb muscle

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ROTTO, DIANE M., CHARLES L. STEBBINS, AND MARC P. KAUFMAN. *Reflex cardiovascular and ventilatory responses to increasing H^+ activity in cat hindlimb muscle.* J. Appl. Physiol. 67(1): 256–263, 1989.—Static exercise increases arterial pressure, heart rate, and ventilation, effects which are believed in part to arise reflexly from a metabolic stimulus in the working muscle. In anesthetized cats, we tested the hypothesis that intra-arterial injections of lactic and hydrochloric acid, which created levels of these substances in muscle similar to those seen during contraction, reflexly increased cardiovascular and ventilatory function. Hydrochloric acid (32 and 57 mM; 1 ml) injected into the arterial supply of the triceps surae decreased intramuscular pH from 7.26 ± 0.05 to 7.17 ± 0.05 ($P < 0.01$) and reflexly increased arterial pressure (23 ± 7 mmHg; $P < 0.01$), heart rate (11 ± 2 beats/min; $P < 0.001$), and ventilation (187 ± 72 ml/min; $P < 0.05$). Static contraction of the triceps surae decreased intramuscular pH from 7.28 ± 0.06 to 7.13 ± 0.06 ($P < 0.01$). Lactic acid was more potent in causing reflexes than was equimolar HCl. For example, lactic acid containing 4 mM lactate and 0.87 mM H^+ reflexly increased arterial pressure, heart rate, and ventilation, whereas 0.87 mM HCl did not. Intra-arterial sodium lactate (13 and 33 mM) at a neutral pH had no effect on these variables. We conclude that contraction-induced accumulation of H^+, especially that arising from lactic acid, might provide a metabolic stimulus to evoke reflex autonomic effects.

exercise; lactic acid; hydrochloric acid; thin fiber muscle afferents; reflex control of the autonomic nervous system

STATIC EXERCISE is well known to increase arterial pressure, heart rate, and ventilation. There is substantial evidence in humans that part of these increases are caused by a reflex arising from exercising muscle. For example, electrically induced static contractions, which are reported not to be painful and which are believed to eliminate central command, have been shown to increase heart rate and arterial pressure with time courses and magnitudes that were almost identical with those evoked by voluntary contractions of equal force (1, 6). In addition, pressor responses to static handgrip have been reduced by half after forearm paralysis with either lidocaine or succinylcholine (2, 5). The remaining half of the pressor response to attempted handgrip in paralyzed subjects was presumed to be caused by central command.

The two types of stimuli most likely to cause the reflex component of the autonomic adjustments to static exercise are mechanical distortion in the contracting muscles and the production of a contraction-induced metabolite. Mechanical stimuli, such as tendon stretch and sudden muscular contraction, have been shown to increase reflexly mean arterial pressure, cardiac rate, and contractility, as well as renal sympathetic nerve discharge (15, 21). By contrast, the reflex autonomic effects of several metabolic products of muscular contraction are unknown.

Recently, we have shown that femoral arterial injections of lactic acid, but not equimolar sodium lactate at a neutral pH, stimulated group III and IV fibers with endings in the triceps surae muscles of anesthetized cats (11). In addition, Stebbins and Longhurst (16) have shown a direct correlation between the magnitude of the reflex pressor response to contraction of the hindlimb muscles and the magnitude of the increase in the H^+ activity in the femoral venous blood. These findings raise the possibility that increases in H^+ activity in working skeletal muscle are a stimulus that evokes the reflex pressor response to contraction. To test this possibility, we have examined in anesthetized cats, the cardiovascular and ventilatory effects of injecting into the arterial supply of the triceps surae and gracilis muscles hydrochloric acid, lactic acid and sodium lactate at a neutral pH. We have paid particular attention to evoking reflex responses to injection of these substances in amounts that simulate the concentrations of H^+ and lactate found in skeletal muscle when it is contracting.

METHODS

Twenty-eight cats were anesthetized with ketamine hydrochloride (20 mg/kg im) followed by α-chloralose (40 mg/kg iv) and urethan (250 mg/kg iv). Additional doses of chloralose (10 mg/kg iv) and urethan (50 mg/kg iv) were given as needed. The cervical trachea, right external jugular vein, and right common carotid artery were cannulated. In some experiments, a gracilis artery was cannulated in a retrograde manner. The tip of the catheter was advanced past the junction of the gracilis and femoral arteries and was stopped when it was positioned ~3 cm from the triceps surae muscles. In these experiments, the femoral and obturator nerves were cut and the hindlimb was skinned. The ankle was ligated tightly and the skin placed back over the hindlimb to cover it. In addition, the femoral vein of the skinned hindlimb was cannulated.

In experiments in which we injected acidic solutions

into the arterial supply of the triceps surae muscles, we also statically contracted this muscle group for 1 min. To contract the triceps surae muscles, we first performed a lumbar and sacral laminectomy, identified the L_7 and S_1 ventral roots and then stimulated electrically (40 Hz; 0.1-ms pulse duration) the cut peripheral ends of these roots with current intensities that were 2.5 times motor threshold. Tension developed by the contracting triceps surae muscles was measured by attaching the cut end of the calcaneal tendon to a force transducer (Grass FT-10).

In other experiments, a catheter was placed in a femoral artery caudal to its junction with the gracilis artery. The tip of this catheter was then advanced so that it was positioned at the junction of the gracilis and femoral arteries. Care was taken to insure that the catheter did not occlude blood flow through the gracilis artery. When the femoral artery was clamped just rostral to the catheter tip, acidic solutions (see below) could be injected into the arterial supply of the gracilis, pectineus, and adductor muscles of the hindlimb. In these experiments, the sciatic and femoral nerves were cut and the femoral vein was cannulated in the hindlimb in which gracilis arterial injections were made.

Expired tidal volume was calculated breath by breath by integrating (Gould) airflow, which, in turn, was measured by attaching a heated pneumotach (Fleisch model 0) to the tracheal cannula. The pneumotach was connected to a Validyne differential pressure transducer (model DP45-16). Intramuscular pH was measured with an ion-sensitive combination needle electrode (20 gauge; 0.9 mm OD; WPI, model SA-4) having a response time of 3 s. The needle electrode, which was connected to an Orion digital pH meter (model SA 520), was inserted 0.5–1.0 cm below the surface of the gastrocnemius muscle or the gracilis muscle. In some experiments, the resting intramuscular pH was below 6.95, a value presumably the result of surgical trauma and cellular damage done by insertion of the needle electrode. In these cases, we corrected the low pH by injecting sodium bicarbonate solution (10%) into the muscle. The injections were distributed over several sites in the muscle and were made with a small (30 gauge) needle. The volume of each injection was 0.05 ml. The injections were ended when the intramuscular pH was between 7.2 and 7.4.

Arterial pressure was measured by connecting the carotid arterial catheter to a Statham transducer (P23 ID). Heart rate was calculated beat to beat from the arterial pressure pulse (Gould Biotach). Arterial pressure, heart rate, tidal volume, and tension generated by the contracting triceps surae muscles were recorded on a Gould ES 1000 chart recorder. Intramuscular pH was recorded manually every 10 s for 1 min before a manuever and for 2 min after its initiation.

Protocols. In the first group of cats ($n = 8$), we measured the decrease in intramuscular pH evoked by static contraction of the triceps surae muscles. We then attempted to evoke a reflex increase in arterial pressure, heart rate, and ventilation by injecting hydrochloric acid solutions into the arterial supply of the triceps surae muscles. We used the contraction-induced decrease in intramuscular pH as a limit when attempting to evoke reflex responses to injecting hydrochloric acid solutions. In this set of experiments our goal was to determine if an injection-induced decrease in intramuscular pH to a level similar to that caused by contraction could evoke reflex increases in arterial pressure, heart rate, and ventilation. In the second group of cats ($n = 7$), we attempted to evoke reflex cardiovascular responses by injecting acidic solutions into the gracilis artery. We selected this artery because it perfuses skeletal muscle, but not joints, bone, or skin.

In the third group of cats ($n = 10$), we examined the effects on arterial pressure, heart rate, and ventilation of injecting hydrochloric and lactic acid solutions into the arterial supply of the triceps surae muscles. In all cases, the volume of injection was 1 ml. Hydrochloric acid was injected in concentrations of 0.87, 3.5, 32, and 57 mM and contained no lactate. Lactic acid was injected in H^+ concentrations of 0.87 and 3.5 mM. The 0.87 mM solution contained a 4 mM concentration of lactate. Likewise, the 3.5 mM solution of lactic acid contained a 19 mM concentration of lactate. A YSI lactate analyzer (model 23L) was used to measure the lactate concentration of the solutions. All lactate concentrations are expressed in terms of the L-isomer. The order in which lactic and HCl acid were injected was varied randomly. All injections were flushed into the arterial supply by 0.5 ml of normal saline. Injections required 25–35 s to complete.

In the fourth group ($n = 3$), we inhibited the activity of both erythrocyte and skeletal muscle carbonic anhydrase. Inhibition of the activity of this enzyme enabled us to determine if the reflex cardiovascular and ventilatory responses to injection of acidic solutions were caused by H^+ or by CO_2. Acetazolamide (60–100 mg/kg) was injected intravenously to block erythrocyte carbonic anhydrase activity. Sodium cyanate (7–14 mg/kg) was injected into the arterial supply of the triceps surae muscles to block skeletal muscle carbonic anhydrase activity (13). Blood from the femoral vein was sampled to show that venous P_{CO_2} increased in response to arterial injection of acidic solutions before blockade of carbonic anhydrase activity but did not change from control levels when acidic solutions were injected after blockade. Blood gas values were measured on a Radiometer ABL3 analyzer. For the 28 cats used in this study, arterial pH, arterial P_{CO_2}, and arterial P_{O_2} averaged at the start of the experiments 7.39 ± 0.01, 31 ± 1, and 103 ± 3 Torr, respectively.

Data analysis. For mean arterial pressure, heart rate, intramuscular pH, and tension generated by the contracting triceps surae muscles, we compared the baseline level with the peak response to either stimulation of the ventral roots or to injection of acidic solutions. For expired minute volume of ventilation, we compared the sum of the tidal volumes for 1 min before contracting the triceps surae muscles or injecting acidic solutions with the sum of the tidal volumes for 1 min immediately after initiation of these manuevers. Onset latencies were measured from the start of injection. All values are expressed as means ± SE. We used repeated measures

analysis of variance followed, where needed, by Scheffé post hoc tests to determine statistical significance. The criterion level for statistical significance was $P < 0.05$.

RESULTS

Reflex responses to injecting hydrochloric acid into the arterial supply of the triceps surae muscles. In eight cats, we first determined the magnitude of the decrease in intramuscular pH when the triceps surae muscles were statically contracted for 60 s. We found that static contraction decreased intramuscular pH from a resting value of 7.28 ± 0.06 to 7.13 ± 0.06, a peak value measured at the 60th s of the contraction period ($P < 0.01$). Intramuscular pH at 10 and 30 s after the onset of static contraction averaged 7.23 ± 0.06 and 7.18 ± 0.06, respectively. Scheffé post hoc comparisons revealed that the pH value at 30 s but not that at 10 s was significantly different from the resting pH value ($P < 0.01$). Contraction evoked significant increases in mean arterial pressure, heart rate, and ventilation (Fig. 1*A*, Table 1). The peak tension developed by the statically contracting triceps surae muscles averaged 7.2 ± 1.0 kg.

In these eight cats, we then injected 1 ml of either 57 mM ($n = 5$) or 32 mM ($n = 3$) HCl solution into the arterial supply of the triceps surae muscles. We selected these doses of hydrochloric acid because they decreased intramuscular pH to levels similar to those decreased by static contraction. Injection of HCl decreased intramuscular pH from 7.26 ± 0.05 to 7.17 ± 0.05 ($P < 0.025$) and significantly increased mean arterial pressure, heart rate, and ventilation (Fig. 1*B*, Table 1). The onset latencies for the increases in mean arterial pressure, heart rate, and ventilation were almost identical with each other and averaged 10.5 ± 1.5 s. The injection-evoked increases in arterial pressure, heart rate, and ventilation remained

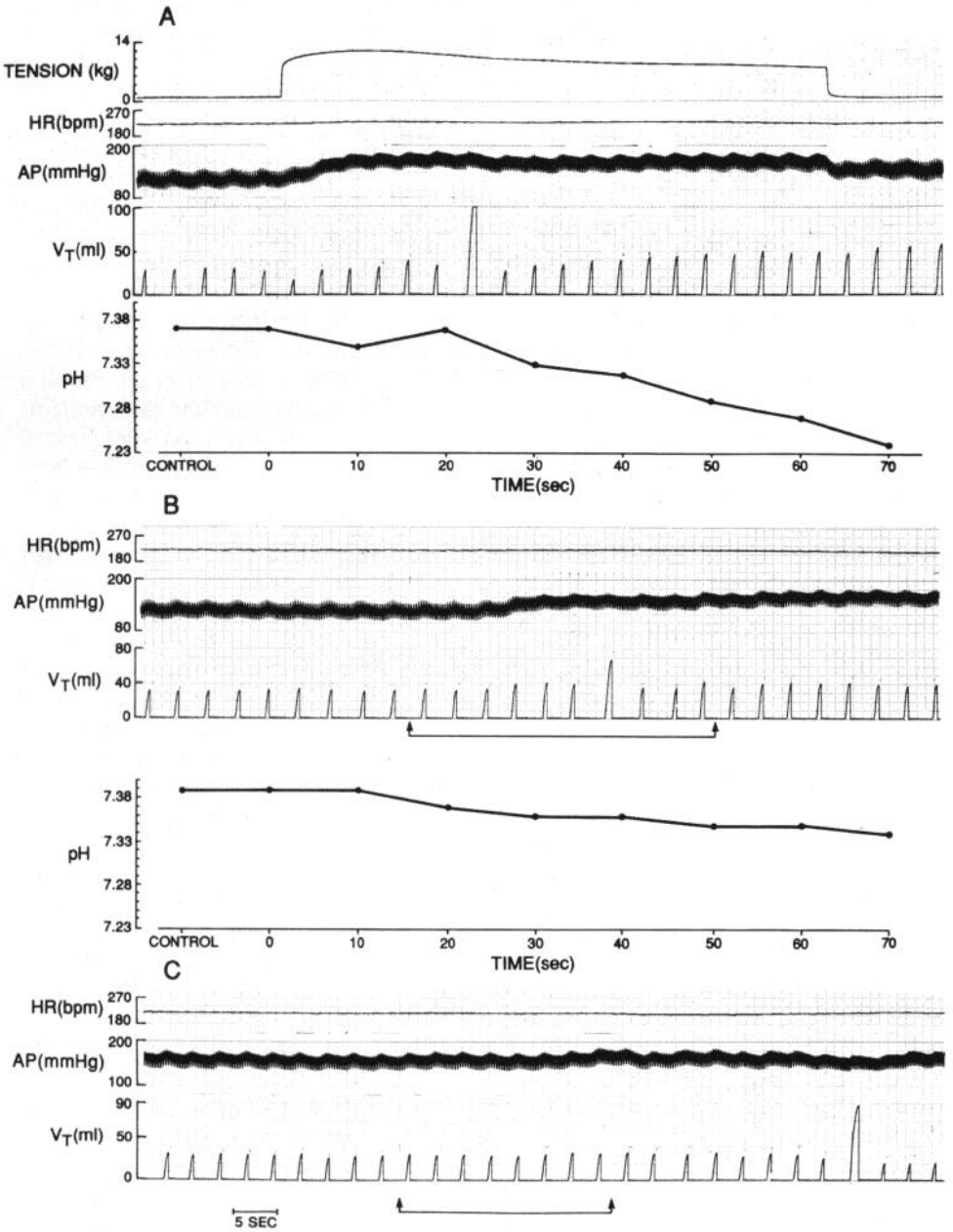

FIG. 1. Effects of static contraction and intra-arterial injection of hydrochloric acid on arterial pressure (AP), heart rate (HR), tidal volume (V_T), and intramuscular pH. *A*, static contraction of the hindlimb muscles increased arterial pressure, heart rate, and ventilation, as well as decreased intramuscular pH. *B*, injection of HCl (32 mM; 1 ml) into the arterial supply of the triceps surae muscles also increased arterial pressure, heart rate, and ventilation, as well as decreased intramuscular pH. Note that the pH decrease caused by injection is within the range of the pH decrease caused by static contraction. *C*, femoral venous injection of HCl (57 mM; 1 ml) had trivial effects on mean arterial pressure, heart rate, and ventilation. Also note that these effects had a much longer onset latency than that evoked by femoral arterial injection in *B*. *A*, *B*, and *C* are consecutive and were obtained from the same cat. Thin line attached at each end to a vertical arrow depicts the period of injection.

TABLE 1. *Effects on mean arterial pressure, heart rate, and expired minute volume of ventilation caused by injecting hydrochloric acid (32 and 57 mM; 1 ml) into the arterial supply of the triceps surae muscles*

	Static Contraction		HCl Injection before Denervation		HCl Injection after Denervation	
	Base line	Peak	Base line	Peak	Base line	Peak
MAP, mmHg	126±9	153±11†	126±7	149±9†	103±9	105±10
HR, beats/min	197±12	206±12‡	195±12	206±13‡	191±11	191±11
$\dot{V}_E$, ml/min	598±95	868±62†	616±74	803±54*	640±65	667±65
f, breaths/min	16±0.9	18±0.6	17±0.4	18±0.5	18±1.1	19±0.9

Values are means ± SE for base-line and peak levels. Measurements were made in 8 cats for mean arterial pressure (MAP) and heart rate (HR) and in 7 for expired minute volume of ventilation ($\dot{V}_E$). Denervation refers to section of the lumbar and sacral dorsal and ventral roots. f, frequency of breathing. Differences statistically significant: * $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$ between base-line and peak values.

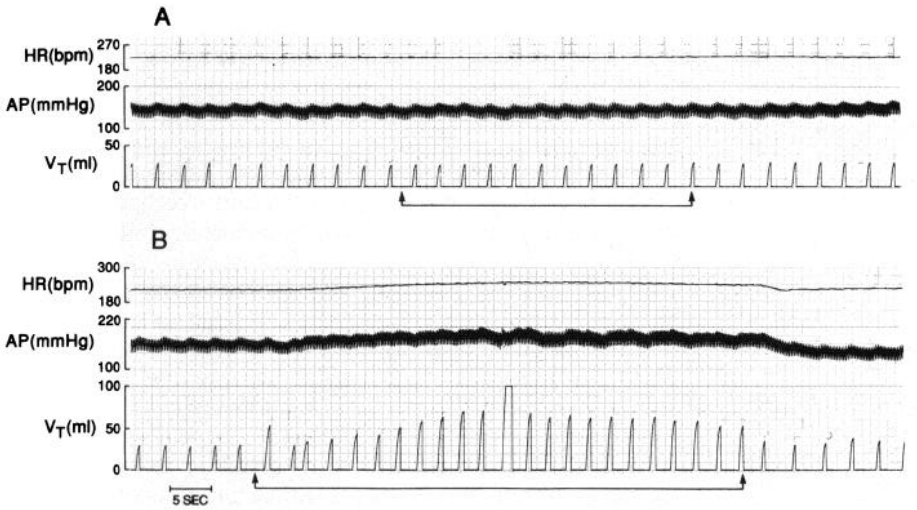

FIG. 2. Effects of electrical stimulation and intra-arterial injection of hydrochloric acid on arterial pressure (AP), heart rate (HR), and ventilation. *A*, injection of HCl (32 mM; 1 ml) into the arterial supply of the triceps surae muscles had no effect on arterial pressure, heart rate, and ventilation after cutting the lumbar and sacral spinal roots. *B*, electrical stimulation (40 Hz) of the central cut end of the L_7 dorsal root increased arterial pressure, heart rate, and ventilation, demonstrating that the preparation was still reactive. The periods of injection (*A*) and electrical stimulation (*B*) are depicted, respectively, by the thin lines attached at each end to vertical arrows. *A* and *B* are consecutive and are from the same cat as that shown in Fig. 1.

above base-line levels for 80.3 ± 11.1, 59.0 ± 10.2, and 81.0 ± 12 s, respectively. The injection-evoked decrease in intramuscular pH remained below base-line levels for at least 3 min. Injection of hydrochloric acid into the femoral vein had either no effect or trivial effects on mean arterial pressure, heart rate, and ventilation in six of the eight cats tested. In the remaining two, femoral venous injections caused increases in mean arterial pressure (6 and 12 mmHg) and ventilation (39 and 320 ml/min), but with onset latencies of 16 and 22 s (Fig. 1*C*). Injection of hydrochloric acid into the arterial supply of the triceps surae muscles after section of the lumbar and sacral dorsal and ventral roots had no significant effects on mean arterial pressure, heart rate, and ventilation (Fig. 2*A*, Table 1).

After section of the dorsal and ventral roots, we found that our preparation was still capable of generating reflex responses. For example, in six of six cats tested, electrical stimulation (40 Hz, 1 ms, 3 mA) of the central cut end of the L_7 dorsal root increased mean arterial pressure and heart rate by 35 ± 13 mmHg and 21 ± 4 beats/min, respectively (Fig. 2*B*). Likewise, in five of these cats, electrical stimulation of the L_7 dorsal root increased ventilation by 436 ± 216 ml/min.

Reflex responses to gracilis arterial injections of HCl. In seven cats, injection of 1 ml of HCl (0.87–32 mM) into the gracilis artery decreased gracilis intramuscular pH from 7.21 ± 0.04 to 7.07 ± 0.07 ($P < 0.05$) and increased mean arterial pressure (from 121 ± 10 to 138 ± 11; $P < 0.01$; Fig. 3*A*). Injection had no significant effect on heart rate, this variable changing from 186 ± 14 to 189 ± 12 beats/min ($P > 0.10$). Ventilation was not measured in these cats. Injection of these doses of HCl into the gracilis vein had no effect on arterial pressure and heart rate (Fig. 3*B*). Cutting the obturator nerve abolished the pressor response to gracilis arterial injection (Fig. 3*C*). Mean arterial pressure averaged 124 ± 7 mmHg before injection and 127 ± 7 mmHg afterward ($P > 0.10$). Likewise, heart rate averaged 193 ± 11 beats/min before injection and 193 ± 12 afterward ($P > 0.10$). After section of one obturator nerve, injection of acidic solutions (1 ml) into the contralateral gracilis artery still increased mean arterial pressure (by 18 ± 3 mmHg) in each of six cats tested (Fig. 3*D*).

Comparison of the reflex effects of lactic acid and hydrochloric acid. In ten cats, we compared the reflex cardiovascular and ventilatory responses to injection into the arterial supply of the triceps surae muscles of lactic acid with those evoked by injection of hydrochloric acid. The H^+ activity of two of the solutions was 0.87 mM, with one containing 4 mM lactate and the other containing 0 mM lactate. The H^+ activity of the two other solutions was 3.5 mM, with one containing 19 mM lactate and the other containing 0 mM lactate. Intramuscular pH was not measured in these cats.

We found that injection of the two acidic solutions containing lactate significantly increased mean arterial pressure, heart rate, and ventilation (Fig. 4*A*). By con-

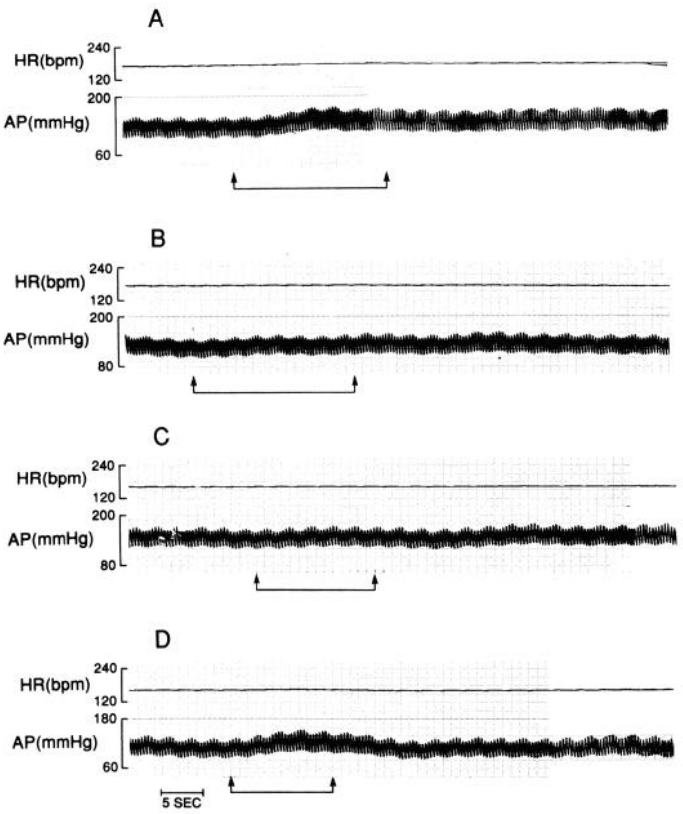

FIG. 3. Injection of HCl into the gracilis artery evokes reflex increases in arterial pressure (AP). *A*, injection of HCl (32 mM; 1 ml) into the left gracilis artery increased arterial pressure. *B*, injection of HCl (32 mM; 1 ml) into the left gracilis vein had no effect on arterial pressure. *C*, injection of HCl (32 mM; 1 ml) into the left gracilis artery after section of the left obturator nerve had no effect on arterial pressure. *D*, injection of HCl (32 mM; 1 ml) into the right gracilis artery still increased arterial pressure. Periods of injection are depicted by the thin line attached at each end to vertical arrows. *A*, *B*, *C*, and *D* are consecutive and are from the same cat. Part of the calibrating grids in *A*, *C*, and *D* were dropped to remove smudges and dirt on the records. HR, heart rate.

trast, injection of the two acidic solutions containing no lactate had no effect on these variables (Fig. 4*B*, Table 2). Femoral venous injection of the lactic acid solutions had no effect on mean arterial pressure, heart rate, and ventilation (Fig. 4*C*). In addition, section of the dorsal and ventral roots abolished the cardiovascular and ventilatory responses to arterial injection.

In five of the ten cats in which we compared the reflex responses to lactic acid injection with those to hydrochloric acid injection, we also examined the cardiovascular and ventilatory effects of sodium lactate (13 or 33 mM; 1 ml) solutions having a pH of 7.56. Sodium lactate (L-isomer) was injected into the arterial supply of the triceps surae muscles and was done before the dorsal and ventral roots were cut. Mean arterial pressure averaged 131 ± 6 mmHg before injection and 131 ± 6 mmHg afterward ($P > 0.10$). Heart rate averaged 228 ± 22 beats/min before injection and 226 ± 22 beats/min afterward ($P > 0.10$). Ventilation averaged 474 ± 135 ml/min before injection and 517 ± 157 ml/min afterward ($P > 0.10$).

In these experiments, injection of either 0.87 or 3.5 mM hydrochloric acid solution into the arterial supply of the triceps surae muscles had no cardiovascular or ventilatory effects. These findings raised the possibility that increases in H^+ activity were not capable of evoking reflex autonomic effects from the hindlimb muscles in this group of cats. Therefore, in seven of the ten cats, we injected 32 mM hydrochloric acid solution (1 ml) into the arterial supply of the triceps surae muscles. We found that injection significantly increased mean arterial pressure (from 138 ± 8 to 171 ± 12 mmHg; $P < 0.001$), heart rate (from 215 ± 23 to 228 ± 23 beats/min; $P < 0.025$), and ventilation (from 557 ± 81 to 989 ± 156 ml/min; $P < 0.01$). Section of the dorsal and ventral roots abolished the cardiovascular and ventilatory responses to 32 mM hydrochloric acid injection into the arterial supply of the triceps surae muscles.

Effect of carbonic anhydrase inhibition. In three cats, blockade of carbonic anhydrase activity with acetazolamide and sodium cyanate had no effect on the cardiovascular and ventilatory responses to injection of 19 mM lactic acid (3.5 mM [H^+]; 1 ml) into the arterial supply of the triceps surae muscles (Fig. 5). Hence, the pressor and tachycardic responses to injection averaged 35 ± 20 mmHg and 10 ± 5 beats/min, respectively, before blockade and 38 ± 9 mmHg and 9 ± 4 beats/min, respectively, after blockade. Likewise, the increases in ventilation evoked by lactic acid injection averaged 467 ± 135 ml/min before blockade and 500 ± 92 ml/min afterwards. Before administration of acetazolamide and sodium cyanate, injection of lactic acid into the arterial supply of the triceps surae muscles increased femoral venous P_{CO_2} by 8.7 ± 4.5 Torr whereas after administration of these carbonic anhydrase blockers, injection of lactic acid increased femoral venous P_{CO_2} by only 1.0 ± 0.6 Torr. The attenuation of the lactic acid-induced increase in venous P_{CO_2} by the carbonic anhydrase blockers occurred in each of the three cats tested. Further evidence of carbonic anhydrase inhibition was the finding that administration of these blockers increased base-line ventilation (from 557 ± 369 to 907 ± 703 ml/min, Fig. 5) and base-line femoral venous P_{CO_2} (from 36.4 ± 4.5 to 41.5 ± 7.2 Torr) in each of the three cats tested. The increase in base-line ventilation caused by inhibition of carbonic anhydrase activity was presumably the result of a decrease in arterial pH.

DISCUSSION

We have shown that injection of acidic solutions into the arterial supply of hindlimb skeletal muscle reflexly increased arterial pressure, heart rate, and ventilation. Moreover, these injection-induced increases appeared to be evoked by decreases in intramuscular pH that were within the range of those found in hindlimb muscle when it was contracted statically. In addition, we have shown that the reflex responses to acidic injection were not dependent on the conversion of H^+ to CO_2 because carbonic anhydrase blockade had no effect on the cardiovascular and ventilatory increases evoked by injection.

The diameter of the intramuscular pH electrode used in our experiments was clearly too large to measure true interstitial H^+ activities. Nevertheless, the resting pH levels that we measured with our electrodes (900 μm) in both the triceps surae and gracilis muscles were similar

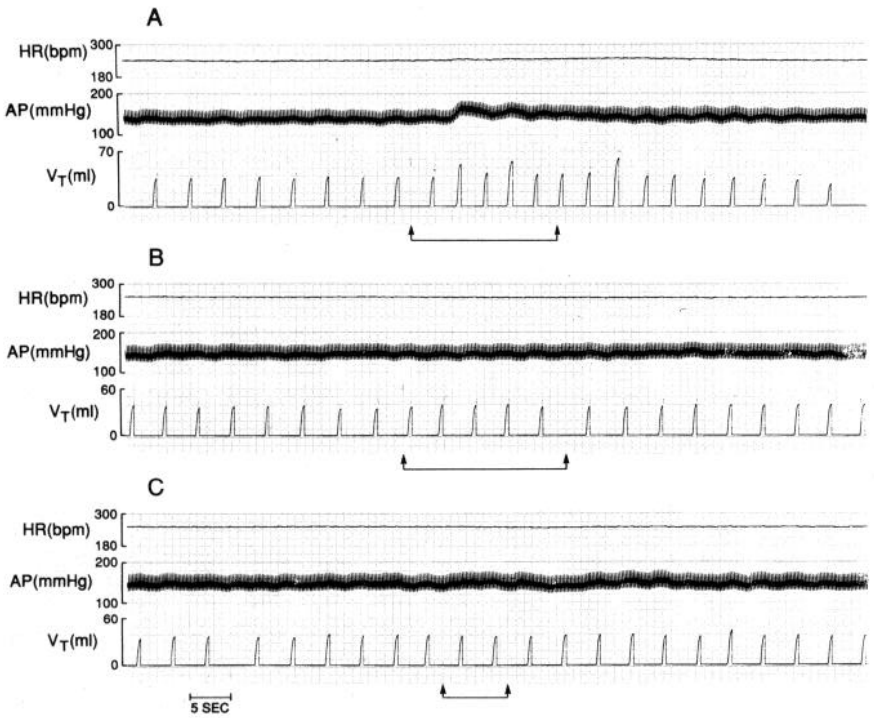

FIG. 4. Injection of lactic acid, but not hydrochloric acid, reflexly increases arterial pressure (AP) and ventilation. *A*, injection of 4 mM lactic acid (1 ml; H^+ concentration = 0.87 mM) into the arterial supply of the triceps surae muscles increased arterial pressure, heart rate (HR), and ventilation. *B*, injection of HCl (1 ml; 0.87 mM) into the arterial supply of the triceps surae muscles had no effect on these variables. *C*, femoral venous injection of 4 mM lactic acid (1 ml) also had no effect. Periods of injection are depicted by thin line attached at each end to vertical arrows. *A*, *B*, and *C* are consecutive and are from the same cat.

TABLE 2. *Effects on mean arterial pressure, heart rate, and expired minute volume of ventilation of injecting into the arterial supply of the triceps surae muscles four solutions, each containing different concentrations of hydrogen and L-lactate ions*

	H^+ = 0.87 mM Lactate = 4 mM		H^+ = 0.87 mM Lactate = 0 mM		H^+ = 3.5 mM Lactate = 19 mM		H^+ = 3.5 mM Lactate = 0 mM	
	Base line	Peak	Base line	Peak	Base line	Peak	Base line	Peak
MAP, mmHg	135±6	144±8*	142±5	143±5	138±7	164±9‡	137±6	141±8
HR, beats/min	220±15	223±15*	221±16	221±15	224±15	229±15†	220±16	221±15
$\dot{V}_E$, ml/min	559±66	661±65†	555±50	543±63	531±68	775±76†	554±69	596±65

Values are means ± SE for base-line and peak levels. Measurements were made in 10 cats. MAP, mean arterial pressure; HR, heart rate; $\dot{V}_E$, expired minute volume of ventilation. Differences statistically significant: * $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$ between base-line and peak values.

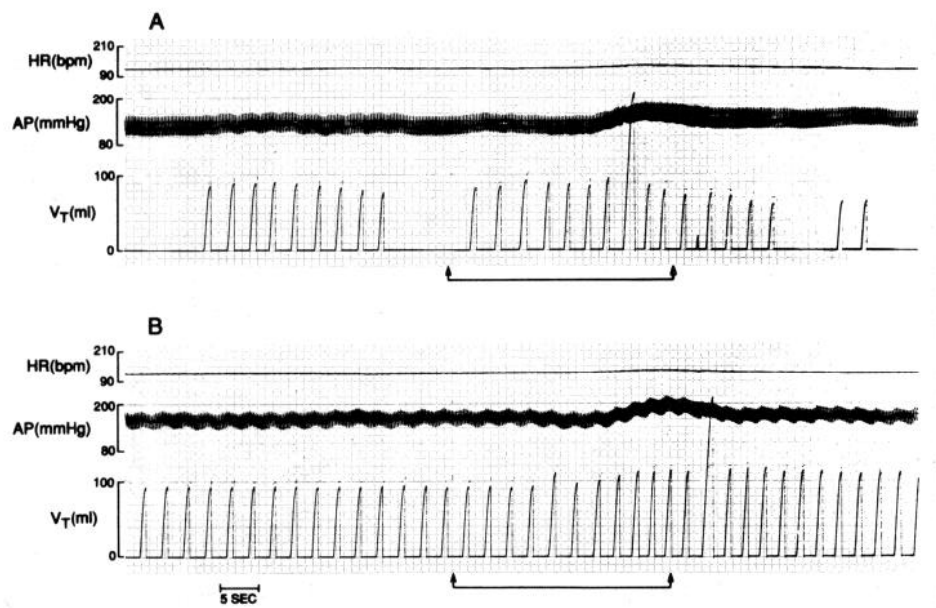

FIG. 5. Carbonic anhydrase inhibition has no effect on the reflex increases in arterial pressure (AP), heart rate (HR), and ventilation evoked by injecting lactic acid (19 mM; 1 ml) into the arterial supply of the triceps surae muscles. *A*: lactic acid injection before carbonic anhydrase inhibition. *B*: lactic acid injection after acetazolamide (60 mg/kg iv) and sodium cyanate (7 mg/kg ia). Note that base-line ventilation increased after carbonic anhydrase inhibition.

to those measured with smaller electrodes (100 μm) in the gastrocnemius muscles of dogs (17). Moreover, the resting intramuscular pH levels that we measured were higher than the intracellular pH of skeletal muscle (9) but lower than the pH of venous blood draining hindlimb skeletal muscle of cats (10) and dogs (17). Our intramuscular pH values were consistent with the well established concept that there is a H^+ concentration gradient from inside the skeletal muscle cell to the venous blood.

In our experiments, lactic acid was far more potent in evoking reflex increases in arterial pressure, ventilation, and heart rate than was equimolar hydrochloric acid. Our findings confirm and extend a report by Thimm et al. (19) who found that lactic acid infusion into the femoral artery of rats reflexly increased heart rate to a greater extent than did hydrochloric acid infusion. We also found that sodium lactate was totally ineffective in evoking reflex responses. These findings suggest that lactate ions, although incapable by themselves of causing reflex cardiovascular and ventilatory increases, are capable of potentiating the reflex responses evoked by increasing H^+ activity in hindlimb skeletal muscle. At present, we can offer no explanation for the mechanism of the lactate potentiation of the H^+ effect.

Although injection of acidic solutions into the arterial supply of the hindlimb has been shown to reflexly increase arterial pressure and heart rate, the source of the afferent limb of the reflex arc has been controversial. Thimm et al. (19), for example, injected lactic acid into the vascularly isolated arterial supply of the hindlimb and speculated that the resulting reflex tachycardia was the result of the stimulation of muscle afferents. Gregory et al. (4), by contrast, reported that the reflex pressor and tachycardic responses to injection of lactic acid into the arterial supply of the vascularly isolated hindlimb were, for the most part, abolished by skinning the hindlimb or cutting the cutaneous nerves. In addition, Gregory et al. (4) reported that these reflex responses to lactic acid injection were still seen after cutting the nerves supplying the muscles of the hindlimb.

Our experiments appear to shed some light on this controversy. The reflex autonomic responses to injection of acidic solutions into arterial supply of the triceps surae muscles were probably caused by stimulation of muscle afferents, but the stimulation of bone and joint afferents might have played some small role in causing these effects as well. Cutaneous afferents, however, could not have played a role in causing these reflex responses because the hindlimb was skinned and the ankle was ligated. Furthermore, the reflex autonomic responses to injection of acidic solutions into the gracilis artery could have been the result of only the stimulation of afferents with endings in the gracilis, pectineus, and adductor muscles of the hindlimb. Our experiments, therefore, indicate that the afferent limb of the reflex pressor response to lactic acid injection can arise from the stimulation of muscle afferents. Our experiments, nevertheless, do not exclude the possibility that lactic acid can also evoke a reflex pressor response arising from the stimulation of cutaneous afferents.

There is substantial electrophysiological evidence that lactic acid is a potent stimulus to group III and IV fibers, the muscle afferents known to comprise the afferent limb of the reflex arc causing the cardiovascular and ventilatory responses to static muscular contraction (7, 8). Graham et al. (3) reported that 2 ml of 100 mM lactic acid injected into the carotid artery of cats stimulated multiunit activity arising from group III and IV afferents with endings in the diaphragm. In addition, Thimm and Baum (18) reported that perfusing the vascularly isolated rat hindlimb with 15 mM lactic acid in tyrode solution stimulated these thin fiber afferents. Last, Rotto and Kaufman (11) have shown that injection of 1 ml of 25 mM lactic acid into the femoral artery of cats stimulated group III and IV afferents. In all these studies, lactic acid appeared to stimulate equal proportions of group III and IV afferents.

At first glance, the concentrations of H^+ in the solutions that we used to evoke reflex autonomic effects might appear to be high. These solutions, however, were injected into arterial blood, which is well known to have an excellent buffering capacity. Moreover, a substantial amount of the injectate might have been swept through the vasculature before it had time to equilibrate with the interstitium. We previously have shown this to be the case for injection of K^+ (12) and speculate that it was also the case for H^+.

Recently, the relationship between changes in intracellular pH in the exercising muscle and increases in either arterial pressure or sympathetic nerve activity have been investigated in humans. Both the time courses and magnitudes of the exercise-induced increases in arterial pressure (14) and sympathetic activity in the peroneal nerve (20) were found to be strongly correlated with the decrease in intracellular pH in the working forearm muscles. Although these elegant human experiments have provided valuable and important insights about the correlation between muscle metabolism and the autonomic responses to exercise, interpretation of their findings is limited because these studies do not allow one to demonstrate a causal relation between increases in skeletal muscle H^+ activity and autonomic effects that are reflex in origin. Our findings in anesthetized cats confirm and extend these correlative findings in exercising humans because they have shown that increases in muscle H^+ activity cause reflex increases in arterial pressure, heart rate, and ventilation. In addition, our findings raise the possibility that the contraction-induced increase in H^+ activity arising from the production of lactic acid might function as an important metabolic stimulus to the thin fiber muscle afferents whose activation causes the exercise pressor reflex.

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