Muscle Cell Electrical Hyperpolarization and Reduced Exercise Hyperkalemia in Physically Conditioned Dogs

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Abstract

Contracting muscle cells release K ions into their surrounding interstitial fluid, and some of these ions, in turn, enter venous plasma. Thereby, intense or exhaustive exercise may result in hyperkalemia and potentially dangerous cardiotoxicity. Training not only reduces hyperkalemia produced by exercise but in addition, highly conditioned, long-distance runners may show resting hypokalemia that is not caused by K deficiency.

To examine the factors underlying these changes, dogs were studied before and after 6 wk of training induced by running on the treadmill. Resting serum [K] fell from 4.2±0.2 to 3.9±0.3 meq/liter ($P < 0.001$), muscle intracellular [K] rose from 139±7 to 148±14 meq/liter ($P < 0.001$), and directly measured muscle cell membrane potential (E_m) in vivo rose from −92±5 to −103±5 mV ($P < 0.001$). Before training, resting E_m of isolated intercostal muscle in vitro was −87±5 mV, and after incubation in 10^{-4} M ouabain, E_m fell to −78±5 mV. After training, resting E_m of intercostal muscle rose to −95±4, but fell to −62±4 mV during incubation in 10^{-4} M ouabain. The measured value for the E_m was not completely explained by the increased ratio of intracellular to extracellular [K] or by the potassium diffusion potential. Skeletal muscle sarcolemmal Na,K-ATPase activity (μM inorganic phosphate mg^{-1} protein h^{-1}) increased from 0.189±0.028 to 0.500±0.076 ($P < 0.05$) after training, whereas activities of Mg^{2+}-dependent ATPase and 5'nucleotidase did not change.

In untrained dogs, exercise to the point of exhaustion elevated serum [K] from 4.4±0.5 to 6.0±1.0 meq/liter ($P < 0.05$). In trained dogs, exhaustive exercise was associated with elevation of serum [K] from 3.8±0.3 to 4.2±0.4 (NS). The different response of serum [K] to exercise after training was not explainable by blood pH.

Basal insulin levels rose from 7.0±0.7 μU/ml in the untrained dogs to 9.9±1.0 μU/ml ($P < 0.05$) after training. Although insulin might have played a role in the acquired electrical hyperpolarization, the reduced exercise-produced hyperkalemia after training was not reversed by blockade of insulin release with somatostatin.

Although the fundamental mechanisms underlying the cellular hyperpolarization were not resolved, our observations suggest that increased Na-K exchange across the sarcolemmal membrane, the increase of Na,K-ATPase activity and possibly increased electrogenicity of the sodium pump may all play a role in the changes induced by training.

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Introduction

Local release of potassium ions from working skeletal muscle cells into the interstitial fluid is thought to play a role in exercise hyperemia and the normal increase of muscle blood flow during exercise (1). Experimental evidence suggests that, during muscle contraction, potassium concentration in interstitial fluid may rise up to 8 (2) or 15 (3) meq/liter. Under conditions of exhaustive exercise, potassium release from contracting muscle may be sufficient to cause transient systemic hyperkalemia (4). The usual rise in serum potassium during exercise does not exceed 1–2 meq/liter (5). However, after prolonged competitive runs, McKechnie and his associates (6) observed serum potassium values approaching 10 meq/liter and corresponding electrocardiographic abnormalities. Thus, exercise hyperkalemia may be of sufficient magnitude to impose risk of cardiotoxicity and potentially fatal arrhythmias. (7) Although it has been observed that training reduces the intensity of exercise hyperkalemia (8), the possible mechanisms whereby this adaptation occurs are unexplored.

In contrast to the foregoing, it is of interest and potential importance that hypokalemia may occur as a consequence of physical training. Resting values for serum K concentration as low as 2.8 meq/liter were observed in men trained to compete in 54-mile races (6). In studying a group of young men before and after track and field training, Rose observed a decline in their average resting serum K concentration from 4.25 to 3.7 meq/liter (9). Some of his subjects showed serum K values below 3.5 meq/liter. Although one might interpret hypokalemia in the highly trained subject to reflect potassium deficiency, there is no apparent reason why endurance training per se should lower cellular potassium stores except in those unusual circumstances in which training is conducted under conditions of extreme heat when large amounts of potassium could be lost in sweat. Previous studies conducted in cool weather (10) on 17 healthy young men showed significantly lower serum potassium values after physical conditioning and an increase of total exchangeable potassium that exceeded the associated gain of lean body mass and total body water. These findings suggest that at least two important electrochemical phenomena may occur in the muscle cells as a result of training. First, one might predict that the concentration ratio of intracellular to extracellular potassium rises. Second, according to the Nernst[1] relationship, the resting potassium diffusion potential (E_K)[2] and in turn, the measured membrane potential of muscle cells

1. $E_k = -61.5 \log (K^i)/(K^o)$, where −61.5 represents the usual constant for RT/F in millivolts, at 37°C, and K^i and K^o represent concentrations of K in intracellular and extracellular water, respectively.

2. *Abbreviations used in this paper:* E_K, potassium diffusion potential; E_m, membrane potential; K^o, serum potassium concentration; P_i, inorganic phosphate.

(E_m) should have increased with the apparent increase of the K^i/K^o ratio (11).

The studies reported herein were designed to explore these possibilities and characterize their mechanisms.

Methods

11 healthy mongrel dogs weighing between 22 and 25 kg were selected for study based upon their ability to run without difficulty on the treadmill. The animals were fed Purina dog chow (Ralston Purina Co., St. Louis, MO) ad libitum. For control studies, the dogs were deconditioned by confinement to their cages for 3–6 wk before studies were initiated. Except for certain pharmacologic interventions to be described, experiments were conducted in each dog before and after training.

Training consisted of treadmill running at a rate of 7 km/h with the treadmill elevated to a 6% grade. Each dog ran for 30 min twice daily, 5 d/wk for 6 consecutive wk. The running program was continued for several additional weeks in some dogs to permit other studies to be described below. To examine the effects of exhaustive exercise on serum potassium, venous blood was obtained from the foreleg immediately before and after an exhaustive run. The latter was accomplished by elevating the angle of the treadmill from the horizontal to a 10% grade and increasing the treadmill speed as much as tolerable for the individual animal. Exhaustion was defined as that point at which the dog either collapsed on the treadmill or refused to run further. Venous blood samples were also analyzed for pH, P_{CO_2}, and P_{O_2} before and after running.

By using techniques described previously (12–14), the difference in resting E_m was measured directly using Ling-type glass electrodes in the gracilis muscle under pentobarbital anesthesia. 12–20 individual fiber potentials were measured in each dog. Values for each animal represent the mean of these individual fiber values. After measuring E_m, samples of muscle were obtained from a different site in the same muscle using a needle biopsy instrument. Water, sodium, chloride, and potassium contents of these samples were determined by methods previously reported from this laboratory (12–15). Total muscle water was partitioned into intra- and extracellular compartments based upon distribution of chloride, which is distributed passively in accordance with electrical potential differences (16). Partitioning of water into intracellular and extracellular components permitted calculation of intracellular electrolyte concentrations.

To avoid effects of pH change or hypoxia on E_m or serum potassium concentration, the animals were ventilated mechanically through an endotracheal tube and monitored to avoid any change of arterial pH, P_{CO_2}, and P_{O_2} during each procedure conducted under anesthesia.

Assays of the Mg-dependent, Na- and K-activated ATPase activity were performed in 10 dogs before and after training. Skeletal muscle microsomal fractions were prepared by the method of Asano et al. (17) with slight modifications. All steps were carried at 0–4°C. A sample of gracilis muscle weighing 0.4–0.8 g was resected, and visible fat and connective tissue were removed by sharp dissection. The muscle was weighed and minced into 6 vol of iced homogenization buffer (mannitol, 250 mM; histidine, 30 mM; Tris-EDTA, 5 mM; Tris deoxycholate, 0.1%, pH 6.8). Employment of Tris-deoxycholate in the incubation mixture, by its detergent action, assured exposure of all enzyme sites to ouabain. The suspension was homogenized in a Polytron (Brinkmann Instruments, Inc., Westbury, NY) at a setting of 6 (medium speed) for 15 s and resheared two additional times with intermittent cooling. The homogenate was centrifuged at 9,000 *g* for 20 min and the supernatants were stored at −60°C overnight. The supernatants were thawed and centrifuged at 100,000 *g* for 40 min in a L5-50 Ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, CA). The pellets were resuspended in 7 ml of medium (mannitol, 250 mM; EDTA, 1 mM; Tris, 125 mM; pH 7.2) by brief homogenization (two or three strokes) in a Teflon-glass Elvehjem-Potter homogenizer and the suspensions were centrifuged at 20,000 *g* for 1 h. The pellets were resuspended in 5 ml of Tris-EDTA (1 mM, pH 7.4) by brief homogenization and the ATPase activity was assayed immediately.

For measurements of Na,K-ATPase and Mg-ATPase activities, 0.2 ml of the resuspended microsomal fraction (50–125 mg of protein) was added to 0.8 ml of medium that contained 0.1 M NaCl, 0.01 M KCl, 0.005 M $MgCl_2$, 1 mM Na-azide, 0.05 M Tris, 0.0001 M EDTA, pH 7.4 (final concentrations), or the same solution devoid of NaCl and KCl or with 0.001 M ouabain added. The tubes were incubated for 4 min at 37°C and the reaction was started with the addition of 0.1 ml of 0.05 M Tris-ATP. The tubes then were incubated for 20 min at 37°C and the reaction terminated by the addition of 0.4 ml of ice-cold 30% trichloroacetic acid. The tubes were kept at 0–4°C for 1–2 h, centrifuged at 5,000 *g* for 10 min, and the liberated P_i determined on 1.0 ml of the supernatant by the method of Fiske and Subbarow (18). The protein content of the trichloroacetic acid precipitates was determined by the method of Lowry et al. (19). These conditions provided linear rates of production of inorganic phosphate (P_i) for 30 min and linear increases in the generation of P_i with protein concentrations up to 150 mg of protein/tube. Mg-ATPase activity was assayed in the medium devoid of Na^+ and K^+ or with added ouabain, and Na,K-ATPase activity calculated as the difference in activity of the system ($+Na^+$, $+K^+$, $+Mg^{2+}$) from that in Mg^{2+} alone or in the presence of ouabain. Both methods, i.e., $\pm(Na^+ + K^+)$ vs. ±ouabain gave similar estimates of Mg-ATPase and Na,K-ATPase activities. Thus, the results reported were based on the ouabain-inhibited portion of the ATPase activity.

Six additional dogs were studied before and after comparable training to determine activity of Ca^{2+}-stimulated ATPase, 5'-nucleotidase, and acetylcholine esterase. For these experiments, skeletal muscle plasma membranes were prepared according to the procedures of Seiler and Fleischer (20) except that the microsomal extraction step also included a 30-s burst of Polytron homogenization at a setting of 6 and the isopycnic sucrose gradient was composed of 50%, 30%, 35%, 23%, and 17% sucrose in 5 mM imidazole, pH 7.4. The Dextran T-10 gradient was omitted (20). Ca^{2+}-stimulated ATPase was measured in the presence of 100 μM $CaCl_2$ with 5 μg of A23187/mg of protein. 5' nucleotidase was measured as described by Morré (21). Acetylcholine esterase activity was measured as described by Seiler and Fleischer (20).

The effect of training on E_m and apparent sarcolemmal ATPase activity was also assessed using the intact, isolated intercostal muscle preparation (22). For this purpose, intact intercostal muscles with their periosteal attachments were removed from six untrained and three trained dogs. Individual muscle fibers were suspended over a paraffin block and continuously perfused with Ringers bicarbonate, equilibrated with 95%:5% O_2/CO_2 and maintained at 37°C. At least 15 potentials were obtained from each specimen. The bathing solution was then replaced with one of identical composition, but containing 1×10^{-4} M ouabain. The potentials recorded after 30 min incubation in ouabain were assumed to reflect inhibition of Mg-dependent, Na,K-ATPase activity.

Insulin is thought to play an important role in potassium dissipation. Hyperkalemia incidental to infusion of KCl causes release of insulin (23), and if glucogenic insulin release is prevented by alloxan, untrained dogs become potassium-intolerant (24). Basal, immunoreactive insulin levels in venous plasma were determined before and after training. These samples were collected after the dogs had fasted 12 h and were unexercised for 24 h. To evaluate the possibility that insulin plays a role in reaccumulation of K into muscle cells after exercise, somatostatin was infused (25) into three trained animals at a rate of 6.7 μg/min beginning 10 min before, during 20 min of exercise, and for an additional 65 min after termination of exercise. Student's *t* test for paired or unpaired data was used for statistical analysis. Data are presented as mean±1 SD.

Results

Resting venous serum potassium concentration (K^o) determined before conditioning averaged 4.2±0.2 meq/liter (range: 3.8–4.4 meq/liter) (Table I). After 6 wk of training, K^o was 3.9±0.3 meq/liter (range: 3.4–4.2 meq/liter, $P < 0.001$). There was no

*Table I. Effect of Exercise Training on Resting Membrane Potential, Serum Electrolytes, Muscle Electrolyte Content, Intracellular Electrolyte Concentrations, and Tissue Water Distribution**

	E_m	K_s	Na_s	Cl_s	K_m	Na_m	Cl_m	K_i	Na_i	Cl_i	TW	ECW	ICW
	−mV	*meq/liter*			*meq/dg FFDS*			*meq/liter ICW*			*ml/dg FFDW*		
x̄±SD													
UT	92±5	4.2±0.2	144±2	103±3	39.8±2.1	12.0±2.1	6.9±1.7	139±7	13.7±5.2	3.5±0.7	335±16	49.0±13.0	286±11
T	103±5	3.9±0.3	144±3	103±7	39.8±2.6	11.9±2.3	6.7±1.1	148±14	12.4±8.0	2.6±0.5	325±10	55.0±11.1	270±16
t	5.5	3.8	—	—	0.8	—	—	3.3	—	—	2.48	—	4.61
P	<0.001	<0.001	NS	NS	NS	NS	NS	<0.001	NS	NS	<0.05	NS	<0.001

* E_m represents the measured resting membrane potential; K_s, Na_s, and Cl_s, the concentrations of electrolytes in serum; K_m, Na_m, and Cl_m, the content of electrolytes in muscle per 100 g fat-free dry solids (FFDS); K_i, Na_i, and Cl_i, the concentrations of electrolytes in intracellular water (ICW); and compartmentation of tissue water content into total water (TW), extracellular water (ECW), and intracellular water (ICW). The symbols UT and T represent untrained and trained. n = 11.

change in concentrations of serum sodium, chloride, or total CO_2 values after training. Before training, an exhaustive run lasted between 20 and 100 min and resulted in a rise in K^o from an average resting value of 4.4±0.5 to a postexercise value of 6.0±1.0 meq/liter ($P < 0.05$). After training, an exhaustive run lasted between 36 and 140 min and resulted in a rise in K^o from an average resting value of 3.8±0.3 to a postexercise value of 4.2±0.4 meq/liter ($P = 0.05$).

The effect of training on venous blood pH and P_{CO_2} (mmHg) before and after exhaustive exercise was as follows: untrained, average values for pH and P_{CO_2} before exercise were 7.35±0.03 and 35±9 mmHg, respectively. After exercise, respective values were 7.47±0.09 and 27±12 mmHg. Trained, average values for pH and P_{CO_2} before exercise were 7.33±0.02 and 42±7 mmHg. After exercise, respective values were 7.44±0.10 and 25±7 mmHg. The differences between these values were not significantly different.

The resting membrane potential of muscle cells before training averaged −92±5 mV and after training, −103±6 mV ($P < 0.001$). Skeletal muscle electrolyte composition data are also shown in Table I. Expressed as milliequivalents per decigram of fat-free dry weight, there was no significant change of either potassium, sodium, or chloride contents. However, both total tissue water content and intracellular water content fell significantly after training, probably reflecting a net increase of muscle solids. Average extracellular water content rose numerically but the difference was not statistically significant.

Intracellular potassium concentration rose in 9 of 11 animals ($P < 0.05$) from an average value of 139±7 to 149±14 meq/liter ($P < 0.01$). Intracellular sodium concentration did not change.

Sarcolemmal Na,K-ATPase activity. Activity of Na,K-ATPase in sarcoplasmic membranes rose uniformly from an average value of 0.189±0.028 (SEM) to 0.500±0.076 ($\mu M\ P_i\ mg^{-1}$ protein h^{-1}). By paired t test analysis, the difference between these means was significant ($P < 0.05$). Magnesium ATPase activity and 5'nucleotidase in sarcolemmal membranes did not change after training (Table II).

Isolated intercostal muscle fibers. Fig. 1 shows the isolated intercostal muscle resting E_m from untrained and trained dogs. The mean E_m in the untrained dogs was −87±5 mV. Incubation in ouabain, 10^{-4} M for 30 min reduced the E_m to −78±5 mV. In the trained dogs, the resting E_m was significantly higher, −95±4 mV ($P < 0.01$). Incubation of trained muscle in 10^{-4} M ouabain resulted in reduction of E_m to −62±4 mV.

Basal immunoreactive insulin levels were 7±0.7 μU/ml in untrained dogs and 9.9±1.0 μU/ml after training ($P < 0.05$). In awake, trained dogs, somatostatin infusion prevented release of insulin after termination of exercise but did not interfere with recovery from hyperkalemia (Fig. 2).

Discussion

These studies show that exercise training consequent to running on the treadmill lowered resting serum potassium concentration, increased the calculated cellular potassium concentration, and increased the measured E_m of skeletal muscle cells. The E_m in the trained dogs exceeded the calculated E_K by 6 mV. That activity of the Mg-dependent, Na,K-ATPase in sarcoplasmic membranes increased more than twofold in the trained animals whereas Mg-ATPase and 5'-nucleotidase remained unchanged suggests that training induced a net increase of pump units per unit mass of sarcolemma. In isolated intercostal muscle cells, ouabain suppressibility of the E_m and the difference between E_m and the E_K (gracilis muscle), increased after training. In the trained dog, hyperkalemia after exhaustive exercise was significantly reduced. The latter observation could not be explained by differences of venous blood pH or P_{CO_2}. Although unproved, such changes could represent a biologic adaptation of skeletal muscle cells for the purpose of defense against potentially dangerous hyperkalemia that might otherwise occur with exhaustive exercise.

Acute hyperkalemia evokes a number of systemic and local physiologic responses that help return potassium into cells or increase potassium excretion into the urine. Those factors promoting cellular uptake of potassium include increased ventilation and respiratory alkalosis (26), catecholamine release

*Table II. Effect of Training on Muscle Enzyme Activity**

	Control	Trained	*P*
Na^+,K^+-activated ATPase	0.189±0.028	0.500±0.076	<0.05
Mg^{2+}-dependent ATPase	0.466±0.092	0.447±0.064	NS
5'-nucleotidase	0.047±0.005	0.051±0.006	NS
Ca^{2+}-stimulated ATPase	0.51±0.26	0.88±0.09	NS
Acetylcholine esterase	8.0±2.4	6.2±1.3	NS

* All data are expressed as $\mu M\ P_i\ mg^{-1}$ protein h^{-1}, ±1 SEM.

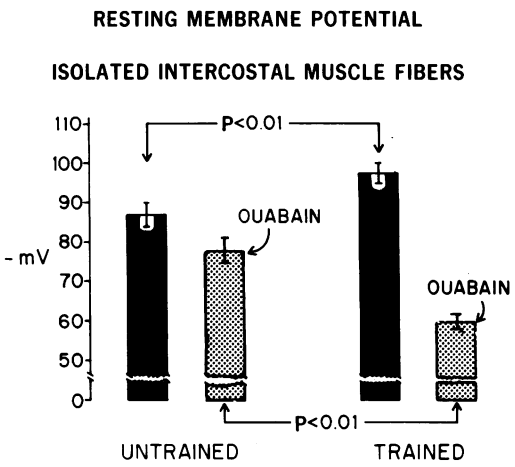

Figure 1. The vertical bars in left portion show the average measured E_m of isolated intercostal muscle fibers from untrained dogs before and after incubation in 10^{-4} M ouabain. In the right side of the figure, the resting E_m was significantly higher after training ($P < 0.01$) and the suppressibility by ouabain was appreciably greater than that observed in untrained muscle cells.

(27), insulin release (24), and cellular depolarization by means of hyperkalemia per se (28). Increasing intracellular sodium concentration during depolarization activates Na,K-ATPase. The resulting extrusion of sodium ions by the pump not only promotes coupled Na-K exchange across the cell, but it also increases cellular electronegativity and thereby promotes uptake of potassium by electrogenic means. Although acute hyperkalemia might cause increased aldosterone production (29), it is doubtful that this response would affect minute-to-minute regulation of serum potassium concentration. Although hyperkalemia may increase excretion of potassium into the urine, such a response would contribute little to immediate serum potassium regulation during severe exercise, because under such conditions, renal blood flow, glomerular filtration, and

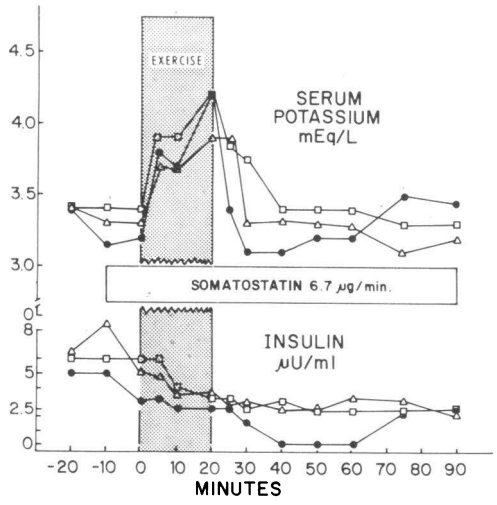

Figure 2. K^o (brachial vein) and insulin levels at rest, during exercise, and recovery. Normally, insulin values fall during exercise but rise sharply when exercise stops, suggesting that insulin may aid in dissipation of exercise hyperkalemia. In trained dogs, prevention of insulin release by somatostatin had no effect on either the appearance or dissipation of hyperkalemia after cessation of exercise.

urine flow are substantially reduced (30). A functionally intact mechanism for kaliogenic insulin release appears to be of critical importance to reduce the intensity of hyperkalemia during potassium infusion (24). However, at least in trained dogs, infusing somatostatin to block insulin release during exercise-induced hyperkalemia did not prevent spontaneous return of K^o to normal (Fig. 2). Thus, it seems likely that other factors must exist at the cellular level that promote dissipation of exercise hyperkalemia independently of insulin. Our findings suggest that local adaptations within the muscle cell were of primary importance.

Brodal and his co-workers (31) have shown that a single bout of exercise in the rat causes an increase of sarcolemmal Na,K-ATPase activity. In animals adapted to a high potassium intake, as well as other situations characterized by increased epithelial secretory capacity for potassium, the increased K-transport capacity has been associated with increased activity of Na,K-ATPase in epithelia of the renal tubule as well as an increase of pump density in the bowel mucosa (32). Moreover, the electrical potential difference between the mucosal surface of the bowel and the extracellular fluid increases after K adaptation. Therefore, even though muscle cells are not epithelial structures, it seems possible that similar phenomena could be operative in skeletal muscle as a result of exercise training.

Within the context of this paper, the potassium regulatory function of the Na,K-ATPase in skeletal muscle is perhaps best discussed in light of certain events that occur during intense muscular work. When muscle cells contract, K is released into the interstitial fluid (33) at a rate such that its concentration in venous blood rises rapidly. Despite continued work at a modest pace, venous K declines, but remains above the level prevailing in arterial plasma so long as work continues. Upon cessation of work, venous K declines further to values less than arterial K and then returns to normal (34).

Potassium release from muscle during exercise presumably results from depolarization of the sarcolemma, and perhaps of more importance, from increased outward conductance resulting from accumulating hydrogen ions produced by metabolism (35). Indeed, the intensity of the initial hyperkalemia is directly related to the pH drop of the muscle effluent (33).

Most of the potassium released from contracting muscle reaccumulates in the cells before it can escape into the venous circulation. Reaccumulation is presumably dependent upon the sodium pump because it can be inhibited by ouabain (34). Although the Na/K pump is oriented in the sarcolemma such that it could be activated by an increased concentration of potassium ions outside the cell and/or an increased concentration of sodium ions inside the cell, there is some question whether the change in extracellular K observed during exercise can actually increase activity of the pump. Although specific kinetic information is not available for the ATPase of skeletal muscle sarcolemma, it is generally agreed that K-stimulated pump activity is maximal at a serum K of 2.5 meq/liter (36). If this is the case, exercise hyperkalemia per se would not be expected to increase pump activity. On the other hand, the efflux of K^+ and H^+ during depolarization and work is associated with influx of Na^+. The range within which intracellular Na increases in the myoplasm during muscular work is indeed capable of increasing pump activity. Studies on skeletal muscle recovering from work showed that pump activity and the sodium transport-dependent consumption of oxygen continue despite the appearance of hypokalemia and

hyperpolarization (34). Hyperpolarization, reflecting the electrogenic effect of the pump, presumably occurs inasmuch as the pump extrudes sodium from the cell at a higher rate than potassium enters. Thus, the charge separation induces a negative potential in the cell (37). Neither hypokalemia, hyperpolarization, nor sodium transport-related oxygen consumption return to normal until myoplasmic sodium falls to its normal concentration. Based upon the foregoing, it can be postulated that myoplasmic accumulation of Na^+ ions during work activates the pump, stimulates Na^+-K^+ exchange, and the resulting electrogenicity of the pump is possibly responsible for additional influx of K that is responsible for postexercise hypokalemia. That reaccumulation of K^+ is not related to a surge of insulin release after exercise in the trained dog is also supported by our observations that exercise-induced hyperkalemia was promptly corrected (even overcorrected in one experiment) despite suppression of insulin release by infusion of somatostatin (Fig. 2).

One of the most interesting and new observations in this study was the electrical hyperpolarization of resting muscle cells in the trained dogs. In general terms, resting electrical hyperpolarization of the muscle cells could occur by disproportionate changes in the relative traffic and resulting concentrations of ions across the sarcolemmal membrane, changes in the activity or stoichiometry of Na-K exchange, or changes in the properties of the sarcolemmal membrane per se. Our findings suggest that hyperpolarization can be explained in part by a change of the E_K. Although muscle K content did not change after training, the intracellular water content (expressed as milliliters of H_2O per decigram of muscle fat-free dry weight) decreased; thereby the intracellular K concentration was higher. Since all available evidence indicates that K activity in skeletal muscle bears a constant relationship to its chemical concentration (38) and that all intracellular K exists in aqueous solution, the Nernst equation may be employed to estimate the E_K. The calculated E_K in the untrained dogs was -93.5 mV. This value is very close to the measured E_M of -92.0 mV. After training, the E_K rose to -97.3 mV, indicating that, although the E_K was probably responsible for at least part of the hyperpolarization, it could not account for the measured E_m of -103.0 mV.

The difference between the E_m and the E_K in the trained dogs, namely 5.7 mV, could represent the electrogenic component of the sodium pump. Although our measurements indicate that overall activity of the sarcolemmal Na,K-ATPase is higher after training, this does not differentiate between an increase in the quantity of enzyme (pump unit density) or a change in the kinetics of the enzyme. If we assume that the properties of the enzyme remained the same after training, then any contribution of the Na-K pump toward a higher resting E_m would depend upon its basal level of activity, which in turn would depend upon an ongoing increased supply of Na^+ ions. Two findings could be explainable by increased Na-K exchange. These include the higher value for intracellular K and the lower value for extracellular K. If the fundamental adaptation in the trained animal consisted of only an increased Na-K exchange across the muscle cell, one would expect to see a decline in the intracellular Na concentration. The fact that intracellular Na remained unchanged is compatible with the view that Na permeability increased, and that the cell increased Na efflux equally. Thereby, the increased rate of Na efflux, and its related electrogenicity, could explain the observed electrical hyperpolarization above the E_K. This could also explain the greater suppressibility of the E_m in the isolated intercostal muscle preparation by ouabain in the trained dogs. Such electrogenicity of the pump would require that the coupling ratio between K^+ entering and Na^+ leaving remained unequal so that charge separation follows.

We observed that basal insulin levels rose by an average of 40% in the trained dogs. Although this change is difficult to interpret, insulin appears to play an important role in sodium-potassium transport in a number of tissues. Insulin receptor density in monocytes increases as a result of training (39). Although the specific effect of endurance training on insulin receptors has not been studied in muscle, studies on skeletal muscle of various species have shown that independently of glucose, insulin hyperpolarizes cells (40), stimulates K uptake (41), increases Na,K-ATPase activity (42), augments [^{3}H]-ouabain binding (43), enhances affinity of pump sites on the inner membrane surface to sodium ions (44, 45), and may multiply pump sites (46). Most recently, evidence has been cited that insulin facilitates Na^+-proton exchange (47), allowing Na^+ to stimulate the pump, which generates electronegativity. Thereby, K^+ ions enter the cell both by Na-K exchange and passively by means of the electrical gradient.

Although blocking further insulin release after exercise in our trained dogs had no effect on recovery from hyperkalemia, insulin release per se after exercise may be unimportant for this purpose. Rather, sustained exposure to increased levels of insulin at the receptor level throughout the day could produce changes facilitating increased passive entry of Na^+ down its electrochemical gradient. In trained dogs, this could serve as a stimulus to induce Na,K-ATPase so that Na^+ entry would be matched by Na^+ efflux and correspond to our observation that intracellular sodium is normal. Even though our calculated value for intracellular sodium was not changed after training, it is possible that sodium concentration in the fluid layer immediately adjacent to the sarcolemma was sufficiently elevated to account for enhanced pump activity. The rheogenic effect of the pump would thereby account for the increased electronegativity of the muscle cell as well as its increased potassium concentration. By this means, a reduction of hyperkalemia in the trained dog after exhaustive exercise could occur by an enhanced rate of potassium flow down an electrical gradient into the muscle cell. In addition, the pump itself could enhance cellular K uptake and hence, retard the intensity of hyperkalemia.

In summary, muscle cell hyperpolarization and increased Na,K-ATPase activity may theoretically explain the reduction of resting K^o and the decrease of hyperkalemia after exhaustive exercise in trained animals.

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