PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 150, 677–680 (1975)

The Role of Potassium Ion in Muscle Glycogenolysis and Glycolysis [1,2] (39104)

J. E. OLERUD, W. H. PRYOR, JR., R. L. EASON, AND H. W. CARROLL
(Introduced by M. S. Silverman)

Naval Medical Field Research Laboratory, Camp Lejeune, North Carolina 28542

Ineffective energy metabolism has been documented as an etiologic factor in exertional rhabdomyolysis (1–3). The importance of potassium ion as a cofactor in enzymatic reactions necessary for normal energy metabolism has been well established (4–7). Since rhabdomyolysis has been associated with potassium deficiency in a variety of clinical situations (8–13), investigators have suggested that the mechanism of rhabdomyolysis in potassium-deficient states may be a functional impairment of enzymes necessary for adequate energy metabolism (8, 14). Two important pathways for muscle energy production are glycogenolysis and glycolysis. Canine muscle homogenate was used in an *in vitro* system to study the effects of potassium deficiency on anaerobic production of lactate from glycogen.

Materials and Methods. Healthy adult mongrel dogs, 14 to 25 kg, were used as study subjects. Potassium deficiency was induced in seven dogs; 11 dogs served as controls. Control dogs received Purina dog food on an *ad libitum* feeding regimen. Potassium deficiency was induced in a similar manner to that described by Knochel *et al.* (15). The potassium-deficient diet (Nutritional Biochemicals Corp., Cleveland, Ohio 44148) contained less than 1 mEq of potassium/kg, but was otherwise nutritionally adequate. To increase kaliuresis, 20 mg/day of desoxycorticosterone acetate was given to each animal for the first five feeding days at an intramuscular site remote from the gracilis.

Determination of muscle potassium level was achieved by performing analyses of sequential biopsies obtained at 1 week prior to starting the deficient diet, 35 days later, and when moderate to severe clinical symptoms of hypokalemia were apparent. Muscle biopsies (0.3 to 0.5 g each) were excised from the suprascapularis, using 1% lidocaine (locally) and xylazine (2.2 mg/kg intramuscularly) for anesthesia.

After the 35-day biopsy, it became apparent that muscle potassium depletion was not severe. To further enhance kaliuresis, 40 to 120 mg/day of furosemide was administered orally to each dog for a period of 1 to 2 weeks. Furosemide was discontinued 1 week prior to the experimental procedures described below. Time required for achieving acceptable potassium deficiency ranged from 46 to 56 days. When the desired deficiency had been achieved, each animal was anesthetized with pentobarbital anesthesia (30 mg/kg intravenously), and gracilis muscles were excised. Muscles were divided into appropriate portions for metabolic and muscle composition studies.

Muscle metabolic studies were performed on gracilis samples which had been frozen immediately after excision from the animal. *In vitro* estimation of anaerobic glycogenolysis and glycolysis was performed in a manner similar to that described by Schmid and Mahler (2). Modifications to assess the effect of potassium ion on those metabolic pathways were as follows: (a) muscle samples were not rinsed in potassium chloride after excision; (b) final magnesium concentration in each incubation flask was 12 mEq/liter; and (c) final potassium concentration was varied by experimental design to range from 0 to 150 mEq/liter in each incubation flask.

[1] The experiments reported herein were conducted according to the principles described in "Guide for the Care and Use of Laboratory Animals" prepared by the Committee for the Revision of the Guide, Institute of Laboratory Animal Resources, National Academy of Sciences–National Research Council.

[2] The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

Endogenous potassium from muscle samples contributed no more than 0.5 mEq/liter to the total potassium concentration of the incubation mixture; (d) 6 mg of purified canine muscle glycogen (16) was the only substrate added to each incubation flask; and (e) Technicon methodology N-61 (Technicon Corp., Tarrytown, New York) was used to determine lactate concentration in the protein-free filtrate of muscle homogenates. Enzyme activity was expressed in μg of lactate produced per mg of protein nitrogen present in the original muscle homogenate. Protein nitrogen was analyzed as described by Ma (17).

Muscle composition studies were performed on 2 to 3 g of fresh lean gracilis muscle dissected free of connective tissue. Water and fat content were determined while preparing muscle samples into a fat-free, dry powder. Water content was determined by drying muscles to a constant weight and using standard gravimetric procedures. Fat was removed from dried muscle by continuous extraction with petroleum ether in a Soxhlet apparatus. Total muscle potassium was determined by wet ashing of fat-free muscle powder in 10% acetic acid. Potassium in the supernatant fluid was assayed by flame photometry. Results were expressed in mEq/100 g of fat-free, dry weight (FFDW). The mean difference in potassium value determined for duplicate muscle samples was 0.9 mEq/100 g FFDW.

Since muscle glycogenolysis and glycolysis occur in the sarcoplasm, an estimate of potassium concentration in the sarcoplasmic fluid was made. A weighed sample of fresh gracilis muscle, 0.3 to 0.6 g, was placed in a Potter–Elvehjem tissue homogenizer containing 10 ml of 0.25 *M* sucrose. The sample was completely homogenized and subjected to ultracentrifugation at 106,000*g* for 30 min. The supernatant portion was analyzed for potassium content. "Sarcoplasmic" concentration of potassium was calculated on the basis of total muscle water content, which was determined from the wet and dry weight measurements of a duplicate sample.

Results. Pronounced deficiency was induced in each of the dogs subjected to the low potassium diet and kaliuretic agents (see Table I). The mean muscle potassium value determined for the seven animals on the deficient diet was 25.2 ± 0.74 mEq/100 g FFDW (mean ± standard error of the mean). The initial mean muscle potassium value for these dogs was 35.9 ± 0.57 mEq/100 g FFDW. The mean muscle potassium value for control animals was 37.4 ± 0.83 mEq/100 g FFDW. Mean serum potassium concentrations were 2.29 ± 0.78 and 4.29 ± 0.46 mEq/liter for the deficient and control dogs, respectively.

Mean sarcoplasmic potassium values for deficient and control gracilis muscles were 74.1 ± 3.4 and 101.7 ± 2.5 mEq/liter, respectively. These results are consistent with those reported by Thiers *et al.* for liver and Hanig *et al.* for brain (18, 19). The correlation between total muscle potassium and sarcoplasmic potassium concentration was $r = 0.88$.

The *in vitro* lactate production by muscle homogenates incubated with various concen-

TABLE I. GRACILIS MUSCLE COMPOSITION.[a]

	Muscle K^+ (mEq/100 g (FFDW))	Sarcoplasmic K^+ (mEq/liter muscle water)
Deficient	25.2 ± 0.74	74.1 ± 3.4
Control	37.4 ± 0.83	101.7 ± 2.5

[a] Values are expressed as mean ± standard error.

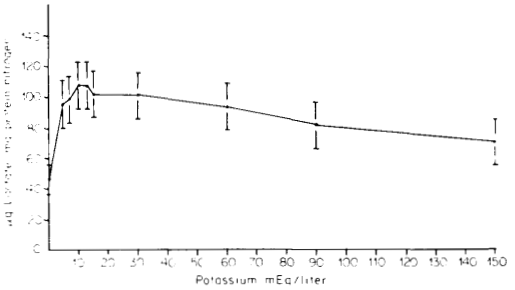

FIG. 1. The effect of potassium ion on *in vitro* lactate production by muscle homogenate. Each point represents mean values for lactate production by muscle homogenate from 11 animals (seven potassium-deficient and four controls). As lactate production by homogenates from control and deficient animals was statistically indistinguishable, the results were pooled. Vertical bars = SEM.

trations of potassium ranging from 0 to 150 mEq/liter is illustrated in Fig. 1. The optimal concentration for glycogenolysis and glycolysis in this *in vitro* system was approximately 10 mEq/liter.

Discussion. This investigation was undertaken to determine whether impairment of glycogenolysis or glycolysis (i.e., ineffective muscle energy metabolism) could be established as a mechanism by which potassium deficiency leads to rhabdomyolysis. A hereditary defect in glycogenolysis has been clearly associated with exertional rhabdomyolysis (1, 2). Furthermore, ineffective energy metabolism has been postulated as the etiology of rhabdomyolysis in a number of clinical situations (20). To determine the effect of potassium deficiency on glycogenolysis and glycolysis in an *in vitro* system, two lines of investigation were followed: (a) determination of potassium concentration necessary for optimal anaerobic production of lactate from glycogen, and (b) estimation of potassium content in the sarcoplasmic fluid of potassium-deficient and control canine gracilis muscle.

The most severely deficient animal in the entire study had a potassium concentration of 59 mEq/liter in the sarcoplasmic fluid. Mean sarcoplasmic potassium values for the deficient and control animals were 74.1 ± 3.4 mEq/liter and 101.7 ± 2.5 mEq/liter, respectively. Fig. 1 shows that not more than 10 mEq/liter of potassium ion is necessary for optimal *in vitro* lactate production from glycogen. Previous observations support the contention that very small amounts of potassium ion are necessary for glycolysis. Ashford *et al.* reported that anaerobic glucolysis proceeded at a significantly higher rate when 5 mEq/liter of potassium was added to an incubation medium containing brain slices (21). Ashford *et al.* and Dickens *et al.* found that addition of 100 mEq/liter of potassium depressed anaerobic lactate production in an *in vitro* brain slice system (21, 22). Based on our observations and those of other investigators, we suggest that intracellular potassium concentrations sufficiently depressed to interfere with anaerobic lactate production would be well below physiologic limits.

Potassium ion has been shown to be important in creatine phosphorylation, necessary for muscle energy transfer. Boyer *et al.* showed that maximum phosphorylation occurred *in vitro* when 200 mEq/liter of potassium ion was added to rat muscle homogenate (6). By extrapolating from Fig. 1 in that paper, one can show that approximately 55% of the maximum phosphorylation rate occurs with as little as 12.5 mEq/liter of potassium ion. Ninety percent of the maximum rate occurs with 75 mEq/liter of potassium. Based on such extrapolations, one might suggest that even with severe deficiency, there would be sufficient potassium ion in the cell water to permit near normal creatine phosphorylation.

Pressman *et al.* demonstrated the importance of potassium ion for mitochondrial respiration *in vitro*. However, enhancement of respiration was maximal at relatively low potassium ion concentrations (25 mEq/liter) (7). Hence, it appears that these enzymatic reactions necessary for aerobic and anaerobic energy metabolism, as well as for energy transfer, require relatively little potassium ion.

Summary. We have presented evidence that in an *in vitro* system, glycogenolysis and glycolysis function normally at potassium levels far below those observed in muscle cell water of severely deficient dogs. We suggest that a functional impairment of glycogenolysis or glycolysis is unlikely to be a mechanism by which potassium deficiency leads to rhabdomyolysis.

The authors wish to acknowledge the invaluable technical assistance of Mr. H. J. Burns, Jr., HM1 C. Causey, Mr. R. Jackson, and Mr. J. Hamby.

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Received July 31, 1975. P.S.E.B.M. 1975, Vol. 150.