ANIMAL MODEL OF HUMAN DISEASE

Defective Skeletal Muscle Glucose and/or Glycogen Metabolism

Animal Model: Defective Skeletal Muscle Glucose and/or Glycogen Metabolism in the Rat

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Biologic Features

Iodoacetate is a sulfhydryl-reactive chemical agent that can be used to selectively inhibit glycolysis.[1] Low concentrations of iodoacetate *in vivo* selectively inhibit the enzyme glyceraldehyde-3-phosphate dehydrogenase, even though other sulfhydryl enzymes may be inhibited *in vitro*.[1-5] Both skeletal and cardiac muscle exposed directly to iodoacetate develop rigor either spontaneously or after several muscle contractions.[2,3,6] Inoculation of the whole animal does not result in rigor. However, large doses produce central nervous system damage with convulsions and death, and smaller doses produce retinal degeneration, testicular damage, cessation of the estrus cycle, hair loss, disturbance of gastrointestinal motility and absorptive function, and renal tubular necrosis (reviewed by Webb [1]). In order to study the effect of iodoacetate on skeletal muscle, one must deliver the drug preferentially to the hind limbs of the animal. Our observations indicate that this method provides an animal model useful for the study of glycogen storage diseases of muscle.

Preparation of the Model

Through a lower midline abdominal incision in an anesthetized rat, a ligature is placed around the aorta below the origin of the renal arteries. Traction is placed on the ligature to occlude aortic blood flow and maintain stability of the vessel. The iodoacetate solution, prepared by dissolving sodium iodoacetate in water and adjusting the pH to 7.4 with sodium hydroxide, is injected into the distal aorta. The concentration of the iodoacetate solution is adjusted such that a dose of 25–28 mg/kg (120–130 μmoles/kg) can be injected in 100–150 μl solution. The aortic ligature is left in place and brought out through the abdominal incision for ischemic exercise; the ligature is removed for rats to be nonischemically exercised. Sixty to ninety minutes after injection, exercise is undertaken.

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Ischemic exercise is produced by delivering supramaximal electrical stimuli, 50 μsec in duration, to the sciatic nerve at a frequency of 5 Hz while simultaneous traction is applied to the aortic ligature. Muscle contraction and relaxation become progressively incomplete, and by 30–45 seconds the foot moves into a fixed hyperplantar-flexed position with the toes spread apart (Figure 1). There is no recordable spontaneous electrical activity in the contractured muscle ("silent cramp").

Nonischemic exercise is achieved by placing the animal in a large tub of water and allowing the animal to swim. The animal will swim vigorously with the fore and the hind legs for approximately 2 minutes, but after 3–4 minutes of swimming both hind legs become stiff, and despite vigorous foreleg paddling, the animal is unable to maintain its head above water. Observable contracture is evident in both hind legs, while there is no evidence of stiffness in the upper limbs.

The contracture resolves within 4–6 hours after nonischemic exercise, while after ischemic exercise the contracture does not resolve for 24 hours. Scintiphotographic scanning with 99mTc-diphosphonate performed 18–24 hours after ischemic exercise shows marked uptake over the contractured muscle (Figure 1). Immediately after exercise, the contractured muscle is hard to palpation and bulges through an incision in the fascia. The muscle has a gray coloration and a mush consistency 24 hours after hard cramp (Figure 1).

Histologic examination of the muscle from a moderately contractured extremity 12–24 hours after exercise reveals necrotic fibers with macrophage infiltration (Figure 1). The necrotic fibers are most numerous in the lateral and medial portions of the gastrocnemius, the tibialis anterior, and the extensor digitorum longus (all muscles highest in Type II muscle fibers), while few necrotic fibers are found in the soleus muscle (which is the highest in Type I muscle fibers). Study of histochemical stains shows the damaged fibers to be the low-oxidative high-glycolytic Type II muscle fibers (the Type IIB fibers).

Comparison With Human Disease

The characteristic features of the diseases of skeletal muscle glucose metabolism (myophosphorylase and phosphofructokinase deficiency) are a painful exercise-induced, electrically silent muscle contracture followed by postexercise rhabdomyolysis, deficient lactate production with ischemic exercise, and absence of striking histopathologic abnormality in unexercised muscle.[7–10] Scintiphotographic scanning with 99mTc-diphosphonate (which follows calcium into damaged muscle fibers) shows excessive uptake into contractured muscle 24 hours after exercise.[11,12] Muscle biopsy specimens from contractured muscle 12–18 hours after ischemic or nonischemic exercise have shown that Type II muscle fibers are selectively damaged.[11] These features of the human diseases are similar to the findings in iodoacetate-injected animals.

Potential Usefulness of the Model

Muscle phosphorylase deficiency is one of the most common of the metabolic muscle disorders.[7] Although the patients appear otherwise normal,

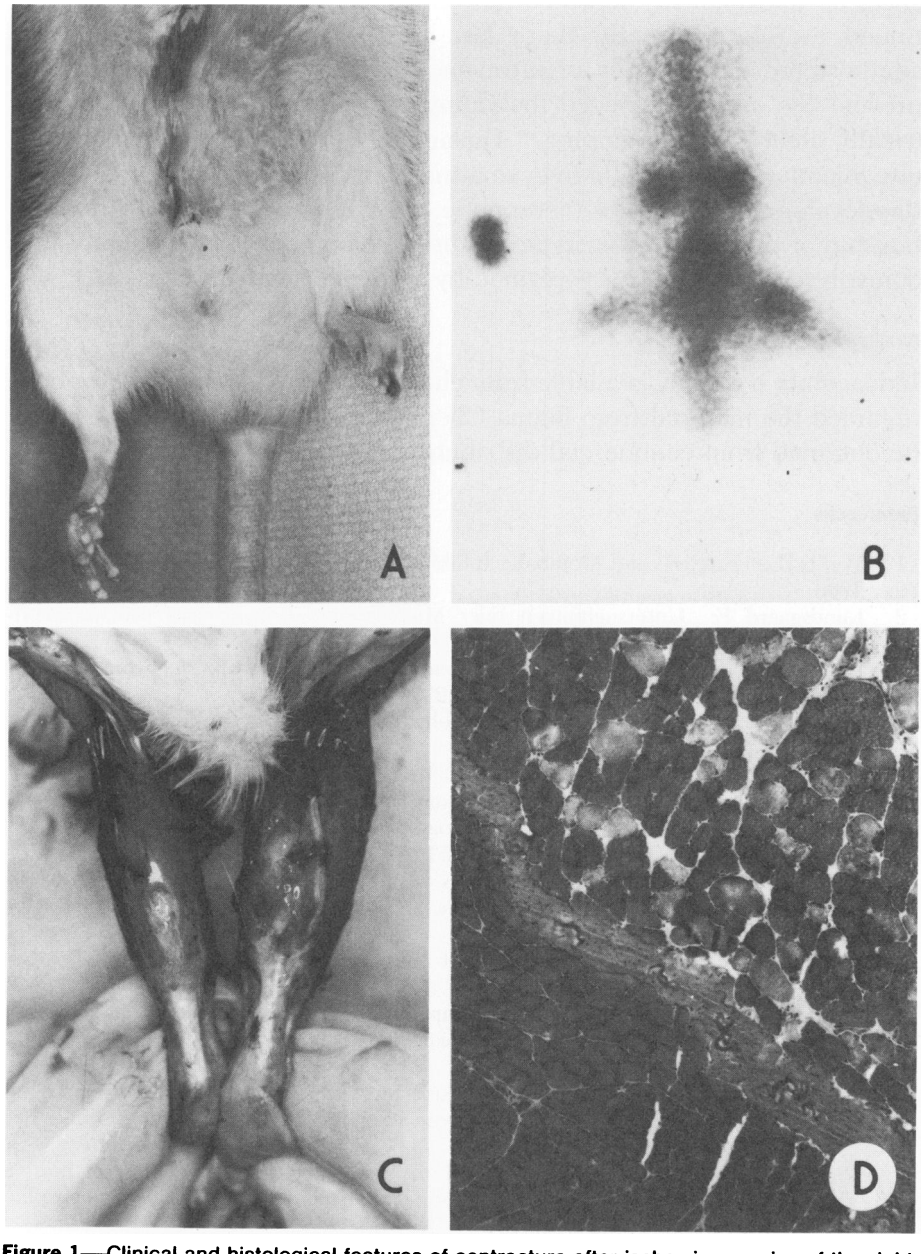

Figure 1—Clinical and histological features of contracture after ischemic exercise of the right leg. **A**—Ventral view of the animal showing the hyperplantar-flexed position of the right leg. **B**—Anterior scintiphotographic image produced by 99m Tc-diphosphonate injection 18 hours after ischemic exercise, showing increased uptake in the contractured right leg musculature (the radioactive marker is on the left side of the animal). **C**—Contractured right gastrocnemius muscle compared with normal muscle in the opposite leg. **D**—Photomicrograph of gastrocnemius (*top*) and soleus (*bottom*) muscles, showing necrotic fibers and macrophage infiltration (modified Gomori trichrome stain).

exercise provokes painful muscle cramps. Marked exercise can induce enough rhabdomyolysis to result in myoglobinuria with fatal renal failure. High glucose and/or fructose diets provide only minimal symptomatic improvement. The patient with muscle phosphorylase deficiency experiences an unexplained "second wind" phenomenon that will permit continued exercise. Recently there has been evidence that triglycerides administered either orally or intravenously at the time of exercise may prevent the exercise-induced rhabdomyolysis without affecting the electrically silent muscle cramping.[13] The animal model produced by iodoacetate injection will be useful in evaluating such clinical findings and permit the development of better therapeutic approaches. In addition, the relationship of the muscle fiber types to the development of cramp and rhabdomyolysis may be better explained by studying this model.

Availability

Iodoacetate is readily available from chemical supply companies (we have obtained the material from Sigma Chemical Co.), and the rats can easily be obtained from commercial distributors.

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