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Isoproterenol-Induced Calorigenesis of Dystrophic and Normal Hamsters (38348)

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(Introduced by R. E. Smith)

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Myopathies such as muscular dystrophy are characterized by alterations in contractile properties of the affected skeletal muscle cells (1–3). Mammalian skeletal muscle, however, in addition to being the vehicle of movement (via contraction) is concerned with another major physiological process—namely, that of heat production. This latter function, which is under the control of the central nervous system, itself reflects two separate pathways in muscle: (a) one involving fiber contraction (*i.e.*, heat production via shivering); and (b) one occurring in the absence of any contraction (nonshivering thermogenesis) (4, 5). Shivering, which consists of oscillatory rhythmic muscle tremors, involves metabolic pathways which appear to differ from those concerned with nonshivering thermogenesis (NST); e.g., NST can be selectively abolished by β-adrenergic antagonists (6, 7) which exert little or no effect on shivering activity. In addition, control of NST is associated with neural pathways which differ from those controlling shivering, NST being mediated by the sympathetic nervous system (6, 7) while shivering is effected by motor efferents.

In view of the probability that these two methods of heat production involve differing cellular pathways as well as differing neural controls, the pathway for NST in dystrophic muscle may be influenced independently of the contractile mechanism. As an initial stage in evaluating possible alterations in NST occurring in dystrophic muscle, the present study compares the nonshivering thermogenic response (measured as isoproterenol-induced calorigenesis (8) of dystrophic and normal hamsters. Since muscle is estimated to contribute about 40% of the NST of normal cold-exposed rodents (9, 10), any significant alteration in the magnitude of the response of dystrophic muscle should be detectable in measurements of the total NST of the intact animal.

Methods. The isoproterenol-induced stimulation of the rate of oxygen consumption ($\dot{V}O_2$) of genetically-conditioned dystrophic male Syrian hamsters (Bio 14.6, aged 90–120 days) was compared to that of normal controls. Five to six days prior to the experiment, the right jugular vein of the hamster was cannulated (PE 10) with the cannula being secured beneath the ventral skin and exteriorized dorsally. The patency of the cannula, which was filled initially with sterile heparinized (40 units/ml) saline, was maintained by flushing with such heparinized saline on alternate days. The animals were caged singly at 22–23° and provided food and water *ad libitum*.

Oxygen consumption was measured five to six days following surgery, at which time the hamsters had returned to their preoperative body weight. For each measurement, the unanesthetized hamster was lightly restrained by placing it in an acrylonitrite holder (Econo-cage) which in turn, was positioned in a water-jacketed chamber open only to a reservoir of 100% oxygen contained in a Med-Sciences Electronics Volume Meter (Model 160). Ambient temperature was maintained at 23.5 ± 0.5° and expired CO_2 was absorbed with Baralyme (barium hydroxide lime) granules. The resting rate of oxygen consumption of the hamster was monitored as he was being infused with a sterile solution of NaCl (0.9%) and ascorbic acid (1 mg/ml). After a resting level had been reached, isoproterenol (DL-isoproterenol-HCl (Sigma), also containing 1 mg ascorbic acid/ml solution) was infused for a period of 60–80 min following which the animal was switched back to the saline–ascorbic acid

infusate. All solutions were infused at a rate of 1.05 μl/min using a Harvard Apparatus infusion/withdrawal pump.

Muscle samples were rapidly frozen (liquid nitrogen/isopentane), sectioned at −20°, air dried and fixed with 10% buffered formalin. These 12 μm sections were stained with hematoxylin and eosin and examined microscopically.

Results and Discussion. Although the hamsters used in this study showed no obvious muscular weakness or impairment of locomotion, histological examination confirmed the presence of the myopathy within the skeletal musculature. Cross-sections of skeletal muscle from the dystrophic animals showed centrally-placed nuclei in almost every fiber (Fig. 1A), whereas fewer than four centrally-placed nuclei/100 fibers is seen in normal skeletal muscle (11; Fig. 1B). In addition, the fiber diameters were somewhat

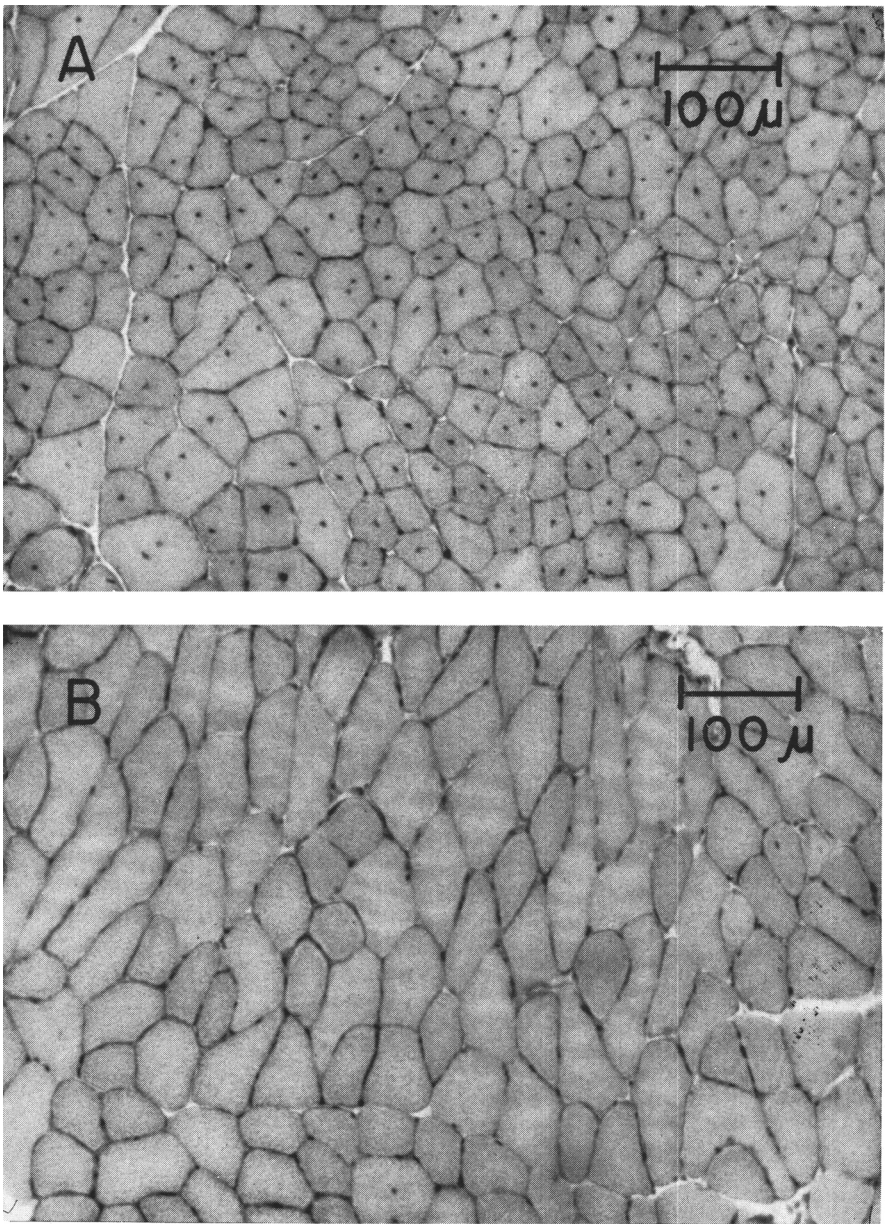

FIG. 1. Cross sections of *anterior tibialis* muscle from a dystrophic (A) and a normal (B) hamster. Sections were stained with hematoxylin/eosin.

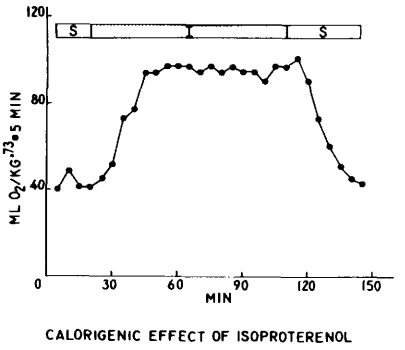

FIG. 2. Temporal pattern of the calorigenic response of a dystrophic hamster to infusion of 1.6 μg/kg·min of isoproterenol.

more variable in the preparations from dystrophic hamsters (Fig. 1A) than were those from the normal controls (Fig. 1B). Despite these aberrations, however, no disruption or necrosis of muscle fibers was evident, nor was any infiltration of connective tissue apparent (Fig. 1A).

Infusion of a given dose of the β-adrenergic agonist, isoproterenol, elicited a calorigenic response which reached a peak within 20–30 min and thereafter remained elevated until the infusate was switched back to saline (Fig. 2). The magnitude of the thermogenesis in the dystrophic as well as normal hamsters was dependent on the concentration of catecholamine infused and appeared to be "saturable" at isoproterenol doses near 10 μg/kg·min (Fig. 3).

At the higher concentrations of catecholamine, the isoproterenol-induced response of the dystrophic hamsters was markedly lower than that of the normal controls. Thus, the maximum rate of oxygen consumption observed (over all doses) was only 20.0 ± 1.7 ml O_2/kg^{-73}·min in the dystrophic hamsters as compared to 27.4 ± 1.1 for the normal controls; however, at the low doses of infusate, no significant differences were evident between the two groups of animals (Fig. 3).

This lack of difference at the lower doses suggests that the depressed responses seen at the elevated concentrations do not simply reflect a decrease in the total number of β-adrenergic receptors (which might have resulted from a decrease in the total number of muscle fibers and/or a decrease in the number of receptors/cell). On the contrary, if the differences observed between the maximum calorigenic response to isoproterenol were due solely to a reduction of functional receptive sites in the dystrophic hamsters, one would expect a depressed response over the entire concentration range rather than at the upper end only. A similar argument can be advanced in support of the view that the depression at high, but not low concentrations of the catecholamine cannot easily be explained by a decrease in the affinity of the receptor for isoproterenol and/or an increase in the rate at which the catecholamine is inactivated at the target cells. Thus, in the dystrophic hamster, alteration of the overall effectiveness of the binding of catecholamine to receptor does not appear to explain the lowered maximum

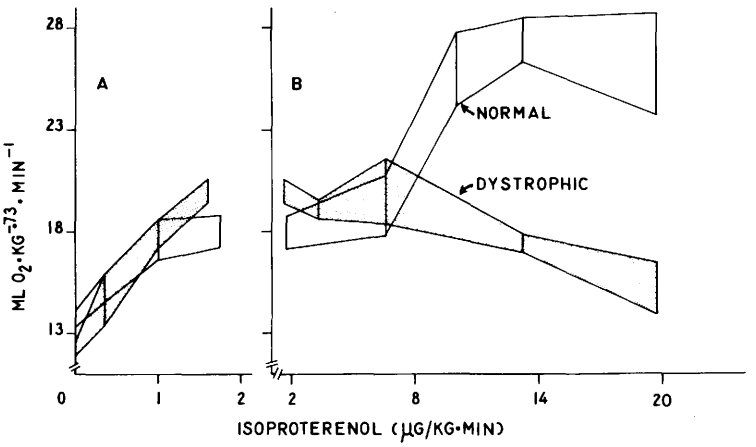

FIG. 3. Calorigenic responses ($\bar{X}$ ± SE) of normal and dystrophic hamsters to various doses of isoproterenol infused at a rate of 1.05 μl/min. Expansion of the abcissa in Graph A shows the overlap at low doses while Graph B illustrates the diverging responses at higher doses.

calorigenic response of the animal to the infused isoproterenol. Nonetheless, the lowered maximum response of the dystrophic animals indicates that their capacity for nonshivering thermogenesis is significantly depressed; and it is likely that in skeletal muscle, alterations occur in the metabolic pathway(s) underlying NST as well as in those involved in the contractile process.

Summary. The calorigenic response of dystrophic hamsters (showing minimal impairment of locomotion or muscle destruction) to the β-adrenergic agonist, isoproterenol, was significantly lower than that of normal hamsters at the higher concentrations (near 10 μg/kg·min) of the infused catecholamine. That differences between dystrophic and normal hamsters did not occur over the entire concentration range suggests that the altered responsiveness of the myopathic animals cannot be attributed solely to a decreased concentration of active catecholamine at the receptor site, a decreased number of functional receptors and/or a decreased affinity of the receptor for isoproterenol. On the other hand, the lowered calorigenic response of the dystrophic hamsters (which appears to reflect a decrease in their ability to generate heat via nonshivering mechanisms) is consistent with the proposed occurrence of alterations in the metabolic pathways associated with NST in skeletal muscle.

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