Shivering and nonshivering thermogenesis in exercised cold-deacclimated rats

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Summary. Norepinephrine (NE)-induced increase in oxygen consumption (\(V_{O_2}\)) and colonic temperature (Tc) was greater in cold-acclimated rats housed at 4°C for 4 weeks (CA) than warm-acclimated controls housed at 24°C for 4 weeks (WA). On the other hand, shivering activity measured at 4°C was less in CA than in WA, while propranolol administration eliminated the difference between these two groups by enhancing shivering in CA. Wet weight and protein content of interscapular brown adipose tissue (IBAT) were greater in CA than in WA. Following cold acclimation, CA were deacclimated at 24°C for 5 weeks. During deacclimation, half of this latter group were forced to run (15 m · min\(^{-1}\) for 1 h) every day (CD-T) while the remaining rats remained sedentary (CD-S). Shivering activity assessed at 4°C 4 weeks after commencing cold deacclimation was significantly less in CD-T than in CD-S and the difference disappeared following propranolol injection. \(V_{O_2}\) and Tc responses to NE injection measured 1, 2 and 5 weeks after commencing cold deacclimation did not differ between CD-S and CD-T. Although IBAT weight was lighter in CD-T than in CD-S, its total protein content was not different between the latter two groups of rats. These results suggest that a greater degree of NE-independent nonshivering thermogenesis (NST) is retained in rats that are exercised during the process of deacclimation as compared with animals that are sedentary. This difference in NST would not seem to be directly related to BAT thermogenic capacity.

Key words: Exercise training — Cold acclimation — Deacclimation — Shivering activity — Norepinephrine response — Brown adipose tissue

Introduction

Exercise training during deacclimation of rats previously acclimated to cold has been reported as retarding the loss of nonshivering thermogenesis (NST) that occurs if there is more than physical inactivity (Moriya 1986). The mechanism and tissues involved in the lasting NST of exercised, cold-deacclimated animals, however, remain unidentified.

It is well known that norepinephrine (NE) is the main mediator of NST in rodents (see review of Landsberg and Young 1983) and that NST occurs largely in brown adipose tissue (BAT) (Foster and Frydman 1978, 1979). In a recent study (Moriya 1986), the change in colonic temperature (Tc) following NE stimulation was measured to indicate the thermogenic capacity (NST) of cold-deacclimating rats. However, Tc measurements are probably not the best index of thermogenic capacity since they are influenced not only by heat production but also by heat loss. Alternatively, the measurement of oxygen consumption (\(V_{O_2}\)) would provide a more precise evaluation of thermogenic capacity during NE stimulation. Thus the present study had as its primary objectives: (i) verification of the effect of exercise training on regulatory thermogenesis in cold-deacclimating rats by measuring \(V_{O_2}\) changes following NE injection and (ii) investigation of BAT properties, indicators of BAT nonshivering thermogenic capacity, to possibly identify a mechanism by which exercise-trained, cold-deacclimating rats potentially retain more NST than sedentary controls.

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In addition, shivering activity upon exposure to cold was evaluated. No previous studies, to our knowledge, have examined shivering in exercised, cold-deacclimating animals. As shivering occurs by muscular contraction (Hemingway 1963; Hothola and Stevens 1986) and since endurance physical activity is known to alter muscle properties (Banchero et al. 1979; Horton 1986; Müller 1976), in a manner which might enhance shivering activity, it seemed pertinent to evaluate shivering activity in exercised, cold-deacclimating rats in order to better determine the mechanism behind their lasting cold adaptability. Thus simultaneous assessment of shivering and nonshivering thermogenic capacity were made, in addition to the evaluation of BAT properties, to provide greater information about thermoregulation in cold-deacclimating animals.

Materials and methods

Animals. Male Wistar rats with a mean initial body weight of 140 g were divided into three groups. One group was housed at 24±1°C for 4 weeks (warm-acclimated rats, WA), a second group at 4±1°C for 4 weeks (cold-acclimated rats, CA) while the third group was reared at 24±1°C for 5 weeks after 4 weeks of exposure to cold (4±1°C) (cold-deacclimated rats, CD). The CD were further divided into two subgroups, sedentary CD (CD-S) and exercise-trained CD (CD-T). Animals were caged individually and kept in their respective acclimation room up to the time of experimentation. The rooms were illuminated from 0700 to 1900 h. Standard commercial laboratory chow (Rodent Laboratory Chow No. 5001, Ralston Purina Co., Indiana, USA) and tap water were provided ad libitum.

WA and CA were used for shivering assessment in the third week of their acclimation period. $V_O_2$ and $T_c$ responses to NE stimulation (NE tests) were determined in the fourth week, approximately 4 days after the measurement of shivering. Approximately 48 hours after NE tests these rats, in the normally-fed state, were killed (between 0830 and 1000 h) by decapitation to permit analysis of BAT.

CD-T were forced to run daily (7 days per week) at 15 m·min⁻¹ for 60 min on a motor-driven treadmill (mdl 2A, Quinton Instruments, Washington, USA) while CD-S were left sedentary in their cages. Shivering activity of CD-S and CD-T was assessed in the fourth week of deacclimation. CD-S and CD-T were given NE tests 1, 2 and 5 weeks after commencing deacclimation and 6—8 rats from each group were killed approximately 48 h after each NE test as described above.

Shivering measurements. Before the evaluation of shivering, animals were adapted to rest quietly in wire cages constructed according to individual body sizes, such that the rats were unable to turn or move excessively once placed in these cages. Once resting quietly, shivering activity was assessed at 24°C for 5 min in all rats and then at 4°C over a period of 360 min in WA and CA or 180 min in CD-S and CD-T. During cold exposure, evaluations were made for 2 min every 30 min in WA and CA or every 15 min in CD-S and CD-T. Shivering activity was assessed from electromyographic activity of the gastrocnemius muscle, using needle electrodes and a dynamograph (model R-411, Beckman Instruments Inc., Illinois, USA). The mean shivering activity was quantified from the product (multiplication) of amplitude and frequency occurring in a 30 s period. Thus values (amplitude x frequency) are averages from each 30 s assessment (e.g. mV·30 s⁻¹). After 270 min of cold exposure in WA and CA or 90 min in CD-S and CD-T, all animals were injected subcutaneously with propranolol (Sigma Chem. Co., St. Louis, USA) (5 mg·kg body weight⁻¹) in order to induce maximal shivering (Rothwell and Stock 1980). Shivering assessment of WA and CA was performed for 270 min when cold before propranolol injection, following the previous experiment (Rothwell and Stock 1980). During cold exposure the activity in WA and CA was found to be nearly constant for 30 to 270 min, so that the duration of cold exposure was shortened to 90 min for CD-S and CD-T in order to decrease the stress on the animals.

$T_c$ was simultaneously measured approximately 15 min prior to cold exposure, immediately preceding propranolol injection and 90 min after the injection, using a thermocouple inserted 5 cm beyond the anus.

$V_O_2$ and $T_c$ were simultaneously measured in order to determine the calorigenic response to NE. Animals were anesthetized with intraperitoneal pentobarbital (Somnotol, M.T.C. Pharmaceuticals, Mississauga, Canada) at a dose of 50 mg·kg body weight⁻¹ and 45 min after the first injection, a second lighter dose of anesthetic (20 mg·kg body weight⁻¹) was subcutaneously administered. NE (L-arterenol bitartrate, Sigma Chem. Co., St. Louis, USA) was intramuscularly injected at a free base dose of 200 μg (in 1 ml of 0.9% NaCl)·kg body weight⁻¹.

$V_O_2$ was measured by an open circuit system with the animals contained in small plastic cages (volume 2,000 ml) within an experimental room at 27±1°C. Air was drawn through the cages at a flow rate of 500 ml·min⁻¹ and pumped into an oxygen analyzer (mdl S-3A1, Thermox Instruments Division, Pittsburgh, USA). $V_O_2$ was calculated by multiplying the air flow through the cages by the difference in oxygen concentration between room air and air from within the cages. Resting $V_O_2$ was measured each 15 min for 45 min, and thereafter the $V_O_2$ response to NE was measured each 15 min for 60 min following the injection. Since the equilibration time of this system was approximately 15 min, the first $V_O_2$ measurements obtained in the resting and NE response conditions were excluded. Therefore, resting $V_O_2$ was calculated as the average of two $V_O_2$ values, the first measurement being excluded.

$T_c$ was measured at rest and 60 min after NE administration as described above.

BAT analysis. Interscapular BAT (IBAT) was dissected and cleaned of adhering muscle, white fat and connective tissue before being weighed. The tissue was then homogenized in ice-cold sucrose buffer solution (pH 7.2) and a sample was used for the determination of total protein content (Schacterle and Pollack 1973).

Statistics. The results are expressed as mean values ± SE of the mean. Data were analyzed by one-way analysis of variance (ANOVA) and the Student t test.

Results

Shivering assessment

Table 1 and 2 show shivering activity (amplitude x frequency) assessed from electromyograph-