Enhancement of Calorigenic Response to Cold and to Norepinephrine in Physically Trained Rats

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Abstract Calorigenic response to cold and to norepinephrine (NE: $4 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) in physically fit (SW) rats was compared with control (CT) and cold-acclimated (CA) rats. Physical fitness was obtained by daily 3-hr swimming in water at 36°C for 6-8 weeks. Resting heart rate (HR) was significantly less in SW rats. Cold immersion (in water at 18°C up to the neck) increased oxygen consumption ($\dot{V}_{\text{O}_2}$) significantly in CA and SW compared to CT rats ($p < 0.01$). Cold immersion decreased colonic temperature (T_{coi}) in all groups. Despite the increased calorigenic response, SW rats cooled as quickly as CT rats in cold water. Body weight and skinfold thickness were significantly less in SW than in CT rats ($p < 0.01$). Compared with CT rats, average weight of the interscapular BAT was significantly less in SW rats ($p < 0.05$). Infusion of NE significantly increased $\dot{V}_{\text{O}_2}$, T_{coi} , HR and mean aortic pressure in anesthetized rats. The increase in $\dot{V}_{\text{O}_2}$ was significantly greater in CA ($p < 0.01$) and SW ($p < 0.05$) than in CT rats. T_{coi} increased significantly in CA ($p < 0.05$) and slightly in SW compared to CT animals.

STRØMME and HAMMEL (1967) observed that physical training produced an increase in metabolic response to cold and in skin temperature on the cold exposed feet, but had no effect on metabolic or thermal responses to norepinephrine in the rat. They concluded that the calorigenic effect of norepinephrine was not increased during cold exposure in the physically trained rats. Since the beneficial changes in muscles in enhancing shivering occurred after physical training (KJELLBERG *et al.*, 1949; PETRÉN *et al.*, 1937; REYNAFARJE, 1962; SCHROEDER *et al.*, 1976; SIEBERT, 1928), the elevation of metabolic rate during cold stress was attributed mainly to an improved shivering capacity. On the other hand, CHIN *et al.* (1973) reported that physical training increased calorigenic response to catecholamines. Although the calorigenic response to infused norepinephrine was not measured, many other reports (GALBO *et al.*, 1977; HARTLEY *et al.*, 1972; ÖSTMAN *et al.*, 1972; VON EULER, 1974) seem to support the observation made by CHIN *et al.*, (1973). Information about the mechanisms responsible for the increased ability to resist cold after physi-

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cal training seems to be conflicting in rats. The present study was undertaken to examine what is responsible for the increased cold tolerance in physically trained rats. To eliminate the unwarranted side effects of hyperthermia or hypothermia, the rats were trained by forced swimming in water at 36°C which kept their body temperature at about 37.6°C throughout the training period.

METHODS

Male rats of the Wistar strain, initially weighing 182 ± 1.6 g, were divided into 3 groups and were kept for 6–8 weeks under the following conditions. 1) Controls (CT): In a temperature-controlled animal room ($24 \pm 1^\circ\text{C}$), the rats were provided with water and a commercial rat chow (Oriental MF, Oriental Yeast Co., Ltd., Tokyo) *ad libitum*. 2) Cold-acclimated rats (CA): In a cold room ($4 \pm 0.5^\circ\text{C}$), the rats were kept in pairs to lessen unwarranted effects of huddling (SUGANO and NAGASAKA, 1979). Water and food were provided as in CT rats. 3) Physically fit rats (SW): The animals, 5 in a group at the first training, were forced to swim for 3 hr in agitated water at 36°C each day (10:00–13:00), which was nearly the same temperature as described by ÖSTMAN *et al.*, (1972). The swimming tanks were $50 \times 50 \times 60$ cm in dimension, and the water was agitated vigorously with a water pump (CP-30B, Hitachi, Tokyo). After swimming, the wet animals were quickly wiped dry with towels and were returned to the animal room in groups. Water and food were provided *ad libitum*. The colonic temperature (T_{col}) of the swimming rats was measured with a thermistor probe during swimming. All these animals were subjected to artificial light from 6:00 to 19:00. At the end of the 6-week acclimation and training periods, the body weights of the rats were 347 ± 7 g for CT rats, 327 ± 14 g for CA rats and 312 ± 11 g for SW rats. The test was made randomly in 3 groups between 6 and 8 weeks, and the average body weights of the rats at the time of the test were 353 ± 12 g for CT rats, 366 ± 7 g for CA rats and 334 ± 15 g for SW rats. The total number of rats tested in this experiment was 46.

Cold tolerance test. One day prior to the test, an individual rat was transferred to the experimental room ($26 \pm 1^\circ\text{C}$), where food and water were supplied *ad libitum*. The rat was confined individually in a $20 \times 20 \times 20$ cm polyvinyl container whose bottom was made of stainless steel wire mesh. The lid of the container was made of plexiglass which had small holes for ventilation. The air temperature inside the container was adjusted to 28°C. After a thermal steady state had been obtained, the rat together with the container was quickly immersed to neck level in agitated water at $18 \pm 0.3^\circ\text{C}$ for 50 min. The head of the rat was kept above water. The residual air in the metabolic chamber was approximately 1,200 ml during cold immersion. The air inside the container was continuously pulled through a hole in the lid with an air pump ($1.5 \text{ liters} \cdot \text{min}^{-1}$). A small fraction of the exhausted air ($180 \text{ ml} \cdot \text{min}^{-1}$) was continuously sent to a Beckman 755 oxygen analyzer and oxygen con-

sumption ($\dot{V}_{O_2}$) was calculated from partial pressure of O_2 and air flow. Before measurement, 3 individual copper wires were sewed on the chest skin under local anesthesia as the electrodes for EKG. EKG were intermittently recorded on a polygraph (Biophysigraph-180, San-ei Sokki, Tokyo) and heart rate (HR) was calculated. T_{co1} was recorded with a thermistor probe inserted to a depth of 7 cm on the polygraph. The rats subjected to 18°C water were not used to measure the responses to norepinephrine.

Calorigenic and cardiovascular responses to norepinephrine (NE). The rat was brought into the laboratory ($24 \pm 1^\circ\text{C}$) and was fitted with polyethylene catheters (0.6 mm outer diameter and 0.3 mm inner diameter, Imamura Rubber Co., Ltd., Tokyo) under urethane ($0.5 \text{ g} \cdot \text{kg}^{-1}$) and chloralose ($40 \text{ mg} \cdot \text{kg}^{-1}$) anesthesia. Aortic and jugular catheters were inserted through the right carotid artery and right jugular vein, respectively. After surgery, the rat was fixed on a stainless steel wire mesh plate and immersed in a warm water bath (*ca.* 36°C) to neck level to keep T_{co1} at around 37.6°C . Since the dose of the anesthetics was high enough to suppress muscle movement throughout the measurement, a tracheostomy was made and the rat was resuscitated with a respirator for small animals (Type 680, Harvard Apparatus, Mass.) to prevent possible depression in respiration by the anesthetics. Expired volume ($\dot{V}_E$) was fixed at $210 \text{ ml} \cdot \text{min}^{-1}$ (tidal volume: 2.2 ml, frequency: 96 min^{-1}). A fraction of the expired air ($180 \text{ ml} \cdot \text{min}^{-1}$) was sent into the Beckman 755 O_2 analyzer *via* a 200 ml bottle reservoir which has a small leak in the cap. $\dot{V}_{O_2}$ was calculated from $\dot{V}_E$ and its partial pressure of oxygen. HR and T_{co1} were measured as described above. Mean aortic pressure was measured with a pressure transducer (Toyo Baldwin, Tokyo) through the aortic catheter and recorded on the polygraph. After a thermal steady state had been obtained, *l*-norepinephrine-bitartrate (Wako Pure Chemical Ind., Osaka) dissolved in saline was infused for 60 min through the jugular vein with an infusion pump (LG-2, Atom Med., Tokyo). The dose of NE infused was $4 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. After the measurements the animals were sacrificed by an injection of saturated KCl solution in the right atrium through the jugular catheter, and weights of the interscapular brown adipose tissue and of the heart were measured. The skinfold thickness was measured at different sites (neck, back and axilla) with a Keys skinfold caliper in CT and SW rats. Weights of the hindleg muscles (tibialis anterior and soleus) were measured in CT and SW rats. All statistical comparisons were done by the Student *t*-test.

RESULTS

Cold tolerance test

Figure 1 illustrates the changes in T_{co1} , $\dot{V}_{O_2}$ and HR during immersion in water at 18°C in CT, CA and SW rats. Following immersion, T_{co1} decreased in all three groups. The rate of fall in T_{co1} was significantly less in CA rats than in the other two groups ($p < 0.05$). Between CT and SW rats, there was no statistical difference

Fig. 1. Changes in T_{col} , $\dot{V}_{O_2}$ and HR during immersion in 18°C water in CT (○), CA (●) and SW (▲) rats. Values are means $\pm$ S.E. Numbers of animals per group are 8, 7, 6 for CT, CA and SW rats, respectively. * $p < 0.05$, # $p < 0.01$: significant difference from the corresponding CT values.

Fig. 2. Responses to infused NE ($4 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) in anesthetized rats. BP denotes mean aortic pressure. Symbols are the same as in Fig. 1. Values are means $\pm$ S.E. Numbers of animals per group are 10, 8, 7 for CT, CA and SW rats, respectively. * $p < 0.05$, # $p < 0.01$: significant difference from the corresponding CT values.

in the rates of fall in T_{co1} . $\dot{V}_{\text{O}_2}$ increased sharply within the initial 10 min of immersion in all groups. The rate of increase in $\dot{V}_{\text{O}_2}$ was greatest in CA rats and least in CT animals. In CA rats the highest $\dot{V}_{\text{O}_2}$ was maintained for the entire period of immersion, while in SW and CT rats the level of $\dot{V}_{\text{O}_2}$ reached 10 min after the onset of cold immersion was not maintained and gradually decreased during the rest of the 50-min immersion. $\dot{V}_{\text{O}_2}$ was, however, significantly higher in SW rats than in CT rats ($p < 0.01$). Resting HR in SW, CA and CT rats were 301 ± 4 , 306 ± 5 and 321 ± 3 bpm at T_a of 28°C , respectively. The resting HR was significantly higher in CT rats than in CA ($p < 0.05$) and SW ($p < 0.01$) rats. In all groups, HR increased sharply in the initial several minutes of immersion and thereafter decreased gradually. The decrease in HR was particularly marked in CT and SW rats. In these groups, HR at the end of 50-min immersion became lower than that observed in the pre-immersion period. HR were highest in CA rats and lowest in SW rats at any time of immersion.

Calorigenic and cardiovascular responses to norepinephrine (NE)

Figures 2 and 3 show the effects of NE infusion ($4 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) on $\dot{V}_{\text{O}_2}$, HR, T_{co1} and mean aortic pressure in anesthetized rats. During the pre-infusion period, the difference in $\dot{V}_{\text{O}_2}$ among the three groups was not significant. After infusion of NE, $\dot{V}_{\text{O}_2}$ increased in all groups. The increases in $\dot{V}_{\text{O}_2}$ at 60 min of infusion period in CA, SW and CT rats were 11.6 ± 1.3 , 7.3 ± 1.2 and $4.4 \pm 0.8 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, respectively. The rate of increase in $\dot{V}_{\text{O}_2}$ was greatest in CA rats and least in CT animals, and the difference in $\dot{V}_{\text{O}_2}$ between these two groups was statistically significant ($p < 0.01$). The increase in $\dot{V}_{\text{O}_2}$ was also significantly higher in SW rats than in CT rats ($p < 0.05$). The increase in T_{co1} with NE infusion in CA, SW and CT rats were 1.50 ± 0.22 , 1.20 ± 0.20 and $0.83 \pm 0.15^\circ\text{C}$, respectively, at 60 min of

Fig. 3. Increases in response to NE ($4 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). Symbols are the same as in Fig. 1. Values are means $\pm$ S.E. * $p < 0.05$, # $p < 0.01$: significant difference from the corresponding CT values.

Fig. 4. Weight of the interscapular brown adipose tissue (ISBAT) and the heart expressed as mg per 100 g of body weight in CT, CA and SW rats. Bars represent means $\pm$ S.E. * $p < 0.05$, # $p < 0.01$: significant difference from the corresponding CT values.

infusion period. The increase in T_{co1} was significantly greater in CA rats than in CT rats ($p < 0.05$). There was no significant difference in T_{co1} between SW and CT rats.

Under anesthesia, HR were nearly the same in three groups in the pre-infusion period. With infusion of NE, HR increased in all groups, but the rates of increase in HR were greatest in CA rats and least in SW rats. Compared with the increase in CT rats, the increases in HR in CA and SW rats were not statistically significant. Mean aortic pressure was significantly higher in SW rats ($p < 0.05$) than in the other two groups in the pre-infusion period. With infusion of NE, mean aortic pressure increased in all groups, but the pressor response to NE was significantly less in SW rats. As shown in Fig. 4, the average weights of the heart and the interscapular brown adipose tissue (BAT) significantly increased in CA rats ($p < 0.01$). In SW rats, the average heart weight increased slightly, but not significantly. The BAT decreased significantly in SW rats ($p < 0.05$). The average skinfold thicknesses in CT and SW rats were 12.7 ± 0.30 and 11.2 ± 0.36 mm, respectively ($p < 0.01$). The weight of hindleg muscles increased significantly in SW rats (tibialis anterior: $195.2 \pm 3.7 \text{ mg} \cdot (100 \text{ g b.w.})^{-1}$ in SW rats as opposed to $176.4 \pm 2.2 \text{ mg} \cdot (100 \text{ g b.w.})^{-1}$ in CT rats ($p < 0.001$); soleus: $39.6 \pm 0.8 \text{ mg} \cdot (100 \text{ g b.w.})^{-1}$ as opposed to $35.7 \pm 0.9 \text{ mg} \cdot (100 \text{ g b.w.})^{-1}$ in CT rats ($p < 0.01$)). These parameters in CA rats were not measured in this study.

DISCUSSION

Our SW rats were physically trained by daily 3-hr swimming at 36°C for 6–8 weeks. According to HARRI and VALTOLA (1975), cold tolerance greatly increased in rats repeatedly forced to swim in cool water at 30°C . In these rats, however, body temperature decreased significantly while swimming. It is difficult to study the effects of physical training *per se* on cold tolerance with such animals. Therefore, the water temperature of 36°C was selected to keep the core temperature of a rat at a normothermic level (about 37.6°C) throughout swimming in this study, as described by ÖSTMAN *et al.* (1972). After the end of the training period, the resting HR in unanesthetized SW rats was significantly lower than CT rats. Many

workers (BARNARD *et al.*, 1976; BOLTER *et al.*, 1973; CREWS and ALDINGER, 1967; TIPTON, 1965) suggested that prolonged periods of endurance training produced a resting bradycardia, which can be employed as a criterion of physical fitness. Since resting HR was significantly lower ($p < 0.01$), it was considered that SW rats in this experiment were apparently physically fit animals.

SW rats cooled as quickly as CT rats in agitated water at 18°C (Fig. 1). However, it would appear that the fall in T_{coi} in CA rats was greatly retarded. Following cold immersion, $\dot{V}_{\text{O}_2}$ was significantly higher in SW rats than in CT animals. At the same T_{coi} , for instance at 34°C, $\dot{V}_{\text{O}_2}$ was significantly higher in SW rats ($36.8 \pm 1.5 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) than in CT animals ($28.5 \pm 1.0 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) ($p < 0.01$) indicating that physical training produced an increase in metabolic response to cold in the rat. Therefore, the rather high rate of fall in body temperature in SW rats could not be attributed to a decrease in calorogenic response to cold. Average body weight of SW rats at the date of test was 334 g, which was approximately 19 and 32 g less than that of CT and CA rats, respectively. The skinfold thickness was significantly less in SW rats than in CT rats ($p < 0.01$). The rather quick fall in body temperature after cold immersion might be largely attributable to the lower body mass and decreased body insulation of SW rats. Since water has a high thermal conductivity, the 18°C water must have been cold for the rats. Whether the same result would be obtained in cool air or during mild cold water immersion needs further investigation. HR increased quickly to certain higher levels within several minutes of cold immersion and thereafter began to decrease. The decrease in HR seems to be dependent on the decrease in body temperature, but the relationship between T_{coi} and HR was different in the physically fit rats compared with those in the other two groups.

Infusion of NE increased HR and $\dot{V}_{\text{O}_2}$ in anesthetized rats (Figs. 2 and 3). The metabolic increase was significantly greater in SW ($p < 0.05$) and CA ($p < 0.01$) rats than in CT animals. T_{coi} increased significantly in CA ($p < 0.05$) and slightly in SW compared to CT rats. These results suggest that physical training by repeated swimming can enhance metabolic response to NE in the rat. Body temperature was kept at about 37.6°C and shivering was not observed throughout the test. The rise in $\dot{V}_{\text{O}_2}$ with infusion of NE may be largely attributed to nonshivering thermogenesis. It is well known that the increased metabolic capacity of the cold-acclimated animals is accompanied by an increased mass of BAT (FOSTER and FRYDMAN, 1978; HELDMAIER, 1974; SMITH and HORWITZ, 1969). HULL and SEGALL (1965) partly excised the BAT in newborn rabbits and found that the calorogenic response to norepinephrine was proportional to the amount of tissue remaining. In CA rats in the present study, the mass of the interscapular BAT increased significantly ($p < 0.01$) and the enhanced calorogenic response to cold and infused norepinephrine seemed to be proportional to the increased mass of this tissue. However, the mass of the interscapular BAT significantly decreased after the daily 3-hr swimming for 6–8 weeks ($p < 0.05$). The participation of the BAT seemed to

be less in the increased calorogenic response to NE in our SW rats. SW rats had a significantly large mass of hindleg muscles (tibialis anterior, $p < 0.001$; soleus, $p < 0.01$). Whether this increased mass of skeletal muscles contributed to the enhanced calorogenic response to NE is not known. The conclusion should be reserved until the metabolic activities of BAT and skeletal muscles have been measured.

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