Capsaicin Increases Endurance Capacity and Spares Tissue Glycogen through Lipolytic Function in Swimming Rats

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Summary The influences of various doses of capsaicin on endurance capacity remain to be clarified. The purpose of this study was to determine whether or not capsaicin delays stored tissue glycogen depletion. Rats were orally given either a vehicle or a dose of capsaicin, 6, 10, or 15 mg/kg of body weight, 2 h before exercise. The rats in each group were divided into three subgroups for resting and swimming exercise (30 min, exhaustion). Swimming exercises were performed with a weight corresponding to 3% body weight attached to the tail, and the endurance capacity was evaluated by the swimming time until exhaustion. The 15 mg/kg dose of capsaicin significantly (p<0.05) enhanced the endurance performance time and plasma concentration of epinephrine, norepinephrine, free fatty acid and glucose rose to significantly higher levels within 30 min; swimming exercise compared to rest (p<0.05). At the 15 mg/kg capsaicin dosage, the plasma insulin level decreased to significantly lower levels in group subjected to 30-min swimming as compared to the resting group (p<0.05), while plasma glucagon rose to a significantly higher level (p<0.05). Liver and gastrocnemius muscle glycogen in the group subjected to 30-min swimming was maintained at significantly higher concentrations in the rats fed 15 mg/kg of capsaicin as compared to the vehicle counterparts (p<0.05). These results suggest that the improvement in swimming endurance with the high capsaicin dosage is caused by an increase in fatty acid utilization as the energy source, resulting in the sparing of glycogen.

Key Words capsaicin, endurance capacity, glycogen, lipolytic function, swimming

Capsaicin (CAP) is a pungent principle in hot red peppers, which are widely used as an important spice for enhancing the palatability of food. It is also utilized in medicine for developing counterirritants (1). Kim et al. (2) demonstrated that the oral administration of CAP successively improved endurance capacity during prolonged exercise trials. The enhanced availability of free fatty acids (FFA) is thought to cause greater fat metabolism in the active muscles, which in turn decreases carbohydrate utilization and leads to increased exercise capacity. However, the influences of CAP on liver glycogen metabolisms during exercise have not been studied in detail. We have recently demonstrated that CAP added to a high-fat diet at 0.014% raised the serum FFA concentrations in rats, which possibly led to an increase in fat utilization as an energy source (3). Kawada et al. (4) reported that CAP administration transiently induced a higher respiratory quotient (RQ), and then markedly lowered it to about 0.75.

The importance of the amount of glycogen stored in working muscles as related to endurance capacity has been described (5). Increased fat utilization during prolonged exercise enables athletes to improve endurance capacity (6). Therefore, the increase in fatty acid utilization during exercise is supposed to improve endurance capacity.

There have been very few studies about the effect of different CAP doses on endurance exercise performance. The relationships between various levels of CAP dosage and metabolic responses during exercise have not been studied in detail. In several published reports, the authors did not measure the responses of liver glycogen and plasma hormone. By examining endurance time to exhaustion, and liver and muscle glycogen metabolism as well as hormonal responses, during prolonged exercise after administering various dosages of CAP, one could gain a deeper understanding how the effects of CAP on exercise performance are mediated. In the present study, we investigated the effects of light, moderate, and heavy doses of CAP on endurance capacity and sparing of stored tissue glycogen in rats subjected to swimming exercise for 30 min.

MATERIALS AND METHODS

Animal care and experimental design. Forty-nine male Sprague-Dawley rats 4 wk of age and weighing 75–85 g were purchased from Japan Charles River Co., Ltd. The animals were housed in individual cages in a room maintained at 24±2°C with 50±5% humidity and a cycle of 12 h of light followed by 12 h of dark. They were fed with a commercial chow diet (Oriental Yeast Co., Ltd., Chiba, Japan) ad libitum and allowed free access to water. Twelve groups treated with different CAP dosages and exercise conditions were investi-
gated regarding circulating hormone, tissue glycogen concentrations, and endurance time to exhaustion: (A) 15 mg/kg capsaicin-treated, sacrificed after exercise to exhaustion (n=6); (B) 15 mg/kg capsaicin-treated, sacrificed after 30 min of exercise (n=6); (C) 15 mg/kg capsaicin-treated, sacrificed at rest; (D) 10 mg/kg capsaicin-treated, sacrificed after exercise to exhaustion; (E) 10 mg/kg capsaicin-treated, sacrificed after 30 min of exercise (n=6); (F) 10 mg/kg capsaicin-treated, sacrificed at rest; (G) 6 mg/kg capsaicin-treated, sacrificed after exercise to exhaustion (n=6); (H) 6 mg/kg capsaicin-treated, sacrificed after 30 min of exercise (n=6); (I) 6 mg/kg capsaicin-treated, sacrificed at rest; (J) vehicle-treated, sacrificed after exercise to exhaustion (n=6); (K) vehicle-treated, sacrificed after 30 min of exercise (n=6); and (L) vehicle-treated, sacrificed at rest (n=6).

For 2 wk before starting the experimental treatment, groups A, B, D, E, G, H, J, and K were allowed to swim 30 min/day to become accustomed to swimming. Thereafter, both swimming and sedentary rats were orally given either the vehicle or CAP at dosages of 6, 10, or 15 mg/kg body weight 2 h before exercise via stomach intubation using a round-ended needle.

On the days of experimental trials, rats allocated to groups A, B, D, E, G, H, J, and K were brought to swim for 30 min or until exhaustion. Groups C, F, I, and L were allowed to rest in their cages for the corresponding time (Fig. 1). The swimming exercise was carried out in a circular tank (50×70×40 cm³) filled with water 55 cm in depth and maintained at a temperature of 36±2°C. The water depth level, 55 cm, was set so the rats could not rest by pressing their tails to the bottom of the tank to support them. Each of the rats had a weight attached (3% body weight) to its tail for the duration of the swimming exercise. This exercise corresponds to an intensity of approximately 4–5 metabolic equivalents (7). The animals were evaluated as exhausted when they failed to rise to the surface of the water to breathe within 7 s. At this moment, the animals were removed from the tank. Swimming workouts were done from 10:00 to 17:00, a period in which minimal variation of the endurance capacity has been confirmed in rats (8), in order to avoid circadian variations in physical activity. Both swimming exercised and resting rats were anesthetized with sodium pentobarbitate and sacrificed by exsanguination from the carotid artery immediately.

The animals were maintained in accordance with the Waseda University Guidelines for the Care and Use of Laboratory Animals.

Measurements and analysis. Blood plasma was collected and stored at −80°C in a deep-freezer for future analysis of catecholamine, glucose, free fatty acid (FFA), insulin, and glucagon concentrations with commercial kits (Wako Pure Chemical Industries). The liver and muscles (white and red gastrocnemius) were removed and then frozen in liquid nitrogen to hold the glycogen intact. The liver and muscle glycogen contents were determined using the Lo method (9). Portions of the muscle and liver were briefly put in a tube containing 1.5 mL of 30% KOH saturated with Na₂SO₄ and immersed in a boiling water bath for 20–30 min. Next glycogen was assayed using commercial kits (Glycogen Test Wako, Wako Pure Chemical Industries).

Statistical methods. Data are expressed as mean±SE. The statistical significance (p<0.05) of differences was determined using two-way analysis of variance (ANOVA) followed by Fisher PLSD post-hoc analysis.

RESULTS

Plasma catecholamine

Figure 2 shows that the 15 mg/kg CAP dose stimulated a greater rise in plasma epinephrine (upper) and norepinephrine (lower) levels after 30 min of swimming exercise as compared to the 6 and 10 mg/kg doses, and increased post-exercise plasma epinephrine to a significantly higher degree than at rest (p<0.05). However, this was not the case for the 6 or 10 mg/kg CAP. The results obtained for norepinephrine were similar, except that the 15 mg/kg CAP raised the level significantly higher than the vehicle even at rest.

Blood energy substrates

In the resting groups, plasma FFA and glucose were not significantly increased by CAP administration. However, after 30 min of swimming exercise, the post-exercise levels of plasma FFA and glucose were significantly higher in the 15 mg/kg CAP group than in the vehicle group (p<0.05). In all the groups treated with CAP, the plasma levels of both energy substrates in the group subjected to swimming exercise for 30 min became higher than those in the resting groups (p<0.05) (Fig. 3).

Hormone

There was no discriminate effect of CAP treatment on the resting levels of plasma insulin and glucagon. In the exercise groups, plasma insulin was maintained at a significantly lower level with the 15 mg/kg CAP dose than with the vehicle (p<0.05) (Fig. 4). In the exercise groups, plasma glucagon rose to a significantly higher level with the 15 mg/kg CAP dose than with the vehicle.
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Fig. 2. Effects of various capsaicin doses and 30 min swimming exercise on plasma of epinephrine (upper) and norepinephrine (lower). Swimming and resting rats were sacrificed by exsanguinations from the carotid artery immediately in the same time. Values are expressed as mean±SE; n=6–7 for each group. Significant difference from corresponding resting group (*p<0.05). Significant difference from corresponding vehicle group (#p<0.05). Significant difference from corresponding vehicle group after exercise until 30 min swimming exercise ($p<0.05).

Fig. 3. Effects of various capsaicin doses and 30 min swimming exercise on plasma free fatty acid (upper) and glucose (lower). Swimming and resting rats were sacrificed by exsanguinations from the carotid artery immediately in the same time. Values are expressed as mean±SE; n=6–7 for each group. Significant difference from corresponding rest group (*p<0.05). Significant difference from corresponding vehicle group after exercise until 30 min swimming exercise ($p<0.05).

Fig. 4. Effects of various capsaicin doses and 30 min swimming exercise on plasma insulin (upper) and glucagon (lower). Swimming and resting rats were sacrificed by exsanguinations from the carotid artery immediately in the same time. Values are expressed as mean±SE; n=6–7 for each group. Significant difference from corresponding vehicle group after exercise until 30 min swimming exercise ($p<0.05).

Glycogen contents

At rest, CAP had no effect on glycogen concentration in the liver or white and red gastrocnemius muscles. Exercise caused a significant reduction in the glycogen content in the liver and white and red gastrocnemius muscles in both the vehicle and CAP groups. Liver and muscle glycogen contents were significantly higher in the CAP (15 mg/kg) group than in the vehicle group after swimming for 30 min (p<0.05) (Table 1).

Effects of swim-to-exhaustion on plasma of catecholamine, FFA, glucose, hormone, liver and muscle glycogen contents

Plasma catecholamine, FFA, and glucagon contents were significantly increased, and plasma insulin and glucose were significantly decreased when rats swam to exhaustion (Fig. 5). Liver and muscle glycogen contents were significantly decreased by the swim-to-exhaustion exercise (Table 2).

Endurance swimming time

The 15 mg/kg CAP group showed a significant increase (219%) in their swimming time to exhaustion as compared to the vehicle group (156±38 min, p<0.05). In the 6 and 10 mg/kg CAP groups, no significant effect of CAP on enhancing swimming performance was ob-
Fig. 5. Effects of various doses of CAP and swim-to-exhaustion on plasma of catecholamine (epinephrine, norepinephrine), FFA, glucose and hormones (insulin, glucagon). Swimming and resting rats were sacrificed by exsanguinations from the carotid artery immediately in the same time. Values are means ± SE. *Significant difference from corresponding each value at rest, p<0.05.

Fig. 6. Comparison of swimming times until exhaustion among three different capsaicin doses. Swimming and sedentary rats were sacrificed by exsanguinations from the carotid artery immediately in the same time. Values are expressed as mean ± SE; n=6–7 for each group. Significant difference from corresponding vehicle group (*p<0.05).

Table 2. Effect of 15 mg/kg dose of CAP on liver and muscle glycogen at swim-to-exhaustion.

|                | Rest  | Exhaustion |
|----------------|-------|------------|
| Liver (mg/g)   |       |            |
| Vehicle        | 55.4±5.0 | 32.9±6.0** |
| CAP            | 69.7±20.0 | 34.1±10.0** |
| White gastrocnemius (mg/g) |       |            |
| Vehicle        | 4.04±0.2 | 0.79±0.3** |
| CAP            | 3.66±0.2 | 1.90±1.0*** |
| Red gastrocnemius (mg/g) |      |            |
| Vehicle        | 4.02±0.3 | 1.15±0.4** |
| CAP            | 3.87±0.1 | 1.52±0.5*** |

Values are means ± SE; n=4–7 for each group.
* Significant difference from rest values, ** p<0.01.
# Significant difference from corresponding vehicle values, p<0.05.

DISCUSSION
Capsaicin (CAP) is known to stimulate lipolysis and/or fat oxidation 2 h after administration. The related hormonal and liver metabolic responses with enhanced lipolysis to graded CAP dosages have not yet been sufficiently investigated. In the present study, we examined the physiological responses of rats to various CAP dose levels. The glycogen sparing effect provides an important survival advantage for improving endurance performance time because glycogen depletion is associated with physical exhaustion and slower glycogen utilization results in improved endurance capacity. The glycogen saved becomes an available energy source for the following exercise phases, which increases endurance capacity. Glycogen concentrations in the liver and muscle, as shown in Table 1, were reduced more...
slowly during a 30-min swimming exercise in the 15 mg/kg CAP group than in the vehicle group. These results of the present study show that oral administration at a dosage level of 15 mg/kg CAP affected the glycogen content in the liver and muscle (white and red gastrocnemius) as well as swimming time during endurance swimming exercise.

Denadai (10) reported that caffeine elevating the FFA plasma concentration decreased the glycogen depletion rate during swimming exercise. This would result in boosting lipid oxidation and depressing muscle glycogen utilization. Fat oxidation is enhanced with increased FFA concentration. In the present study, a significant increase in FFA was observed at a dosage of 15 mg/kg CAP. These results suggest that the glycogen thus saved could become an available energy source for the following exercise phases and that glycogen delays the onset of fatigue.

Catecholamine plays a crucial role in preventing hypoglycemia during swimming exercise (11). CAP significantly increased circulating catecholamine levels and successively improved endurance swimming performance. As shown in Fig. 2, CAP increased the circulating catecholamine levels during exercise only at the dosage level of 15 mg/kg, and did not have an affect at the dosage levels of 6 and 10 mg/kg during a 30-min swimming exercise.

Costill et al. (12) reported the increase in endurance time until exhaustion with a caffeine ingestion. Ivy et al. (13) also reported enhanced exercise performance and found a significant difference in lipid oxidation. In the present study, the endurance time until exhaustion was significantly higher in the 15 mg/kg CAP dosage group as compared to the vehicle group.

Decrements in insulin secretion and increments in glucagon secretion are involved in precisely matching increased glucose production with the increased glucose utilization that prevents hypoglycemia during exercise (14–16). As shown in Fig. 4, plasma insulin secretion was significantly higher in the resting group as compared to the groups subjected to 30-min swimming exercise, but plasma glucagon secretion was significantly higher in the groups that swam as compared to the resting group.

In summary, 6 and 10 mg/kg CAP doses administered 2 h prior to exercise had no effect on endurance performance in untrained rats loaded with swimming exercise at moderate work rates. We propose that a 15 mg/kg dose of CAP acts as a lipolytic agent, giving significant effects on the sparing of tissue glycogen during exercise, because the higher dose enhances lipolytic activity in the adipose tissues that maintain higher plasma FFA concentrations and glycogen. Therefore, a high dosage is a useful ergogenic acid when given 2 h prior to endurance exercise in rats. Further work to elucidate the mechanism by which CAP promotes sparing glycogen is clearly warranted. The data presented here shows that high doses can dramatically alter many physiological and metabolic responses in endurance performance.

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