Increase in Swimming Endurance Capacity of Mice by Capsaicin-induced Adrenal Catecholamine Secretion

Kyung-Mi Kim, Teruo Kawada, Kengo Ishihara, Kazuo Inoue, and Tohru Fushiki

Laboratory of Nutrition Chemistry, Department of Food Science and Technology, Faculty of Agriculture, Kyoto University, Kyoto 606–01, Japan

Received April 21, 1997

Increase in endurance swimming capacity caused by capsaicin (CAP), a pungent component of red pepper, -induced increase of fat metabolism in mice was investigated using an adjustable-current water pool. The mice administered CAP via a stomach tube, showed longer swimming time until exhaustion than the control group of mice, in a dose-dependent manner. The maximal effect was observed at a dose of 10 mg/kg while more than 15 mg/kg had no effect. The increase of endurance was observed only when CAP was administered two hours before swimming. After the administration of CAP, the serum glucose concentration rapidly increased and then decreased within 60 min, while the concentration of serum-free fatty acids gradually increased through 3 hours. The residual glycogen concentration of the gastrocnemius muscle after 30 min of swimming was significantly higher in the CAP-administered mice than in control mice, suggesting that use of the serum free fatty acids spared muscle glycogen consumption. The serum adrenaline concentration significantly increased with twin peaks at 30 min and two hours after administration of CAP. An experiment using adrenalectomized mice was done to confirm that the effect of CAP is due to increased energy metabolism through the secretion of adrenaline from the adrenal gland. The swimming endurance capacity of the adrenalectomized mice was not increased by CAP administration, although adrenaline injection induced a 58% increase in the endurance time. These results suggest that the increase of swimming endurance induced by CAP in mice is caused by an increase in fatty acid utilization due to CAP-induced adrenal catecholamine secretion.

Key words: capsaicin; swimming endurance capacity; adrenaline

Endogenous adrenaline increases adipose tissue lipolysis and intramuscular triglyceride breakdown. The increased fat availability can spare muscle glycogen during aerobic exercise. In contrast, a number of studies have demonstrated that exogenous adrenaline administration increases muscle glycogenolysis during electrical stimulation and aerobic exercise. Therefore, it is not known whether manipulation of the plasma adrenaline concentration improves muscle glycogen depletion and increases performance during endurance exercise.

Indeed, caffeine, for example, has been demonstrated to elevate the plasma adrenaline concentration approximately two-fold, but despite this, caffeine ingestion induces a sparing of muscle glycogen. Either the production of adrenaline does not increase muscle glycogenolysis or, alternatively, more glycogen is spared by use of plasma fatty acids as a fuel than is used up in muscle glycogenolysis. Recently, Chesley et al. demonstrated that muscle glycogenolysis is unaffected by a doubling of the adrenaline concentration during intense aerobic exercise despite elevated levels of phosphorylase. These findings led us to speculate that ingestion of certain foods that can increase endogenous adrenaline secretion may promote a sparing of muscle glycogen and increase endurance capacity.

Among food materials, some pungent principles of spices have been demonstrated to cause the increase of adrenaline secretion from the adrenal medulla. CAP is the major pungent principle in various species of Capsicum fruits, hot pepper, for example, and causes striking pharmacological effects, especially on the primary afferent neurons. We have demonstrated that CAP induced an increase of adrenaline secretion from the adrenal medulla, which was mainly caused through activation of the central nervous system. After the administration of CAP, the respiratory quotient (RQ) transiently increased and then markedly decreased to about 0.75.

The purpose of this study was to find whether CAP ingestion, inducing higher plasma adrenaline concentrations, would improve endurance swimming capacity in mice, and to prove that this effect on endurance capacity, if it would exist, was solely due to epinephrine secreted from the adrenal gland.

Materials and Methods

Materials. CAP was purchased from Fluka AG (Buchs, Switzerland; Lot No. 258397 186). 3,4-Dihydroxybenzylamine (DHBA) and adrenaline were from Aldrich Chemical Co. and Sigma Chemical Co., respectively. Other chemicals were obtained from Nakarai Chemical Co. (Tokyo, Japan) and Wako Pure Chemical Industries (Osaka, Japan).

Animal treatment. Animals. Male Std ddY 6-wk-old mice (Japan SLC, Hamamatsu, Japan) used in the experiment were housed in standard cages (33 × 23 × 12 cm, 6 mice/cage) under controlled conditions of temperature (22 ± 0.5°C), humidity (50%), and lighting (light from 0700 to 1900h). They were provided a stock diet (type MF; Oriental Yeast Co., Ltd., Tokyo, Japan), which consisted of water 80, protein 246, fat 56, and

---

1 To whom correspondence and reprint requests should be addressed.

Abbreviations: CAP, capsaicin; Adex, adrenalectomized; DHBA, 3,4-dihydroxybenzylamine.
carbohydrate 523 (g/kg diet), and water ad libitum. The care and treatment of experimental animals conformed to the Kyoto University guidelines for the ethical treatment of laboratory animals.

Adrenalectomy. The adrenalectomy was done through a dorsal approach under ether anesthesia as described elsewhere, using mice of about 40 g body weight. After the adrenalectomy, mice were given 0.9% NaCl solution instead of water as reported elsewhere and were fed for 7 days until the experiment. Complete removal of the adrenal was confirmed after the experiments by direct observation and analysis of the plasma adrenaline concentration. The operation was found to be successful in 6 of the 8 mice. Swimming performance data from the remaining mice were discarded.

Administration of CAP and vehicle. The mice were administered CAP orally (3-15 mg/kg body weight) via a stomach tube. CAP was suspended in 0.9% NaCl solution containing 3% ethanol and 10% Tween 80 as described elsewhere. The dosage of adrenaline, 0.3 mg/kg, administered intraperitoneally, which increases the serum adrenaline concentration to 20 ng/ml 30 min after injection, was decided upon preliminary experiments with the dose of adrenaline and continuous measurement of serum adrenaline concentration. The mice were administered 400 µl of CAP or a vehicle solution as a control. 1, 2, and 3 hours before swimming. The adrenalectomized mice were given 10 mg/kg CAP or the vehicle 2 hours and 0.3 mg/kg of adrenaline 30 min before swimming. A cross-experiment was also done in the case of adrenalectomized mice.

Current swimming pool. An adjustable current water pool was used for measurement of endurance swimming capacity. The details have been described previously. We used an acrylic plastic pool (90 x 45 x 45 cm), which was filled to a depth of 38 cm with water. The surface of the tank was smooth and clear to prevent the animals from supporting itself while swimming. The current in the pool was generated with a pump (type C-P600H, Hitachi, Tokyo, Japan). Water was returned to the pump through a diameter 55 mm diameter 55 mm pipe sitting in a plastic pipe set on the bottom of the pool. The strength of the current was adjusted by the change of water flow. The water flow was changed by opening and closing the valve and was monitored by a water flowmeter (Ashida Co., Kyoto, Japan). The distribution of surface current speed was measured with a digital current meter (type SPC-5 Sanko Industry Co., Tokyo, Japan) at 12 points of equal intervals on the water surface. The water temperature was strictly maintained at 34°C by a water heater and thermostat. The high reproducibility and sensitivity of this apparatus on the evaluation of the maximum endurance capacity of mice have been reported.

Measurement of maximum swimming time. To avoid circadian variations in physical activity, experiments were done from 11:00 to 17:00, a period in which minimal variation of endurance capacity has been confirmed in mice. After a 1 wk preliminary period in which animals became accustomed to swimming, i.e., 30 min swimming at a flow rate of 6 liter/min a day, maximum swimming time was measured for division of mice into two groups. Animals were assessed to be fatigued when they failed to rise to the surface of the water to breath within a 7 seconds period. A period of longer than 7 seconds resulted in frequent drawings while less than 5 seconds reduced the reproducibility of the test. The mice were divided into 2 groups (CAP and vehicle) with equal mean of swimming time before the experiment. The swimming endurance capacity of the two groups was measured by a cross-experiment and the paired data were analyzed.

Muscle glycogen analysis. Mice were made to swim for 30 min in a current of 7 liter/min, and immediately after the swimming load, the glycogen concentrations of the gastrocnemius muscles were measured by an enzymatic techniques as described. Briefly, glucose residues were determined after hydrolysis of the muscle sample in 1 mol/liter HCl at 100°C for 2 hours, using a commercial kit (Glucose CII Test Wako, Wako Pure Chemical Industries).

Serum fatty acid and glucose analysis. Thirty µl of mouse blood was taken from the tail at 30 min intervals for 4 hours after administration of CAP or vehicles. Serum FFA was measured by an acyl CoA-synthetase and acyl CoA oxidase enzyme method with a commercial kit (NEFA C-Test Wako, Wako Pure Chemical Industries). Glucose was assayed by combination of mutase and glucose oxidase with a commercial kit (Glucose CII, Test Wako, Wako Pure Chemical Industries).

Serum adrenaline analysis. Blood for adrenaline assay was collected from the severed neck veins and the serum was stored at -20°C until assay. The serum sample was purified with aluminum oxide by the method of Anton and Sayre. Serial samples (100 µl) containing 10% Na2S2O3 (50 µl/ml) and DHBA (40 ng/ml) as the internal standard were added to 100 µl of 2% Tris-HCl buffer, pH 8.6, and aluminium oxide (100 mg/ml). The mixtures were shaken in a microtube mixer for 10 min, the supernatant was removed, and the aluminium oxide was washed twice with methanol and distilled water. The adrenaline was eluted with 60 µl of 0.5 N HCl. The eluate was assayed by an HPLC-electrochemical detector as described.

Data analysis. Data are expressed as means ±SEM. Comparisons of swimming capacity between the means of two groups were done by the paired Student's t test. The data on adrenaline and the metabolic parameters were analyzed by the unpaired t test. Statistics were calculated with the INSTAT software package (Macintosh Version 3.00, GraphPad Software Inc., San Diego, CA). A level of p < 0.05 was used as the criterion for statistical significance.

Results

Effects of CAP on swimming endurance capacity

CAP (6 mg/kg body weight) or vehicle solution was orally administered to mice 30 min, 1, 2, and 3 hours before the start of the swimming experiment and the time to fatigue in a flow rate of 7 liter/min (19 cm/s, surface current speed) was measured. As shown in Fig. 1, the endurance of mice administered CAP 1 or 3 hours before swimming was not increased, while the mean swimming time of the group at 2 hours increased 13 min (about 40%) as compared with vehicle-administered mice (p < 0.01, the paired t test). Mice given CAP 2 hours before swimming showed a longer swimming time than other mice. Swimming endurance capacity 2 hours after CAP administration was maximized at a dosage of 10 mg/kg. No further increase was observed at 15 mg/kg (Fig. 2).

The change of serum adrenaline concentration following administration of CAP

Serum adrenaline concentration was measured after oral administration of CAP (10 mg/kg). The adrenaline concentration in serum was maximized within 2 hours, and then

![Graph of Increased swimming endurance vs Time of measurement](image_url)

Fig. 1. Effects of CAP on Swimming Capacity. CAP (6 mg/kg) or vehicle solution were orally administered 30 min, 1, 2, or 3 h before start of swimming in a current strength of 7 liter/min. The swimming capacity of mice was measured by cross-experiments and analyzed by a paired t test. The average swimming time of control mice administered vehicle solution were 67.4 ± 8.8 min (30 min), 45.2 ± 5.6 min (1 h), 38.6 ± 3.8 min (2 h), and 37.7 ± 7.0 min (3 h) (means ± SEM). Values are means ± SEM for 11-25. * Significantly different from control, p < 0.01.
Fig. 2. Effects of CAP Concentration on Swimming Capacity.
CAP or vehicle solution were orally administered 2h before swimming in a current of 7Inter/min. The swimming capacity was measured by cross-experiments and analyzed by a paired t test. The mean swimming time of control mice were 42.3 ± 9 min (3 mg), 42.4 ± 8.1 min (6 mg), 54.1 ± 9.1 min (10 mg), and 46.9 ± 7.5 min (15 mg) (means ± SEM). Values are means ± SEM for 7-10. * Significantly different from control, p < 0.05 (vs. placebo group).

Fig. 3. Change of Serum Adrenaline Concentration by CAP Administration.
The serum adrenaline concentrations were analyzed at various times after oral administration of CAP (10 mg/kg) to conscious mice. Blood of mice administered CAP was collected by decapitation. The data were analyzed by an unpaired t test. Values are means ± SEM for 3-8. * Significantly different from placebo, p < 0.01.

Fig. 4. Course of Serum Fatty Acid Concentration after CAP Administration.
CAP (10 mg/kg) were administered to mice under anesthesia. Blood was taken from the tail at 1h intervals and the data analyzed by an unpaired t test. Values are means ± SEM for 3-12. Significantly different from control. * p < 0.05, ** p < 0.01.

Fig. 5. Course of Serum Glucose after CAP Administration.
CAP (10 mg/kg) or vehicle solution were orally administered under anesthesia. Blood was taken from the tail at 30 min or 1h intervals. The data were analyzed by an unpaired t test. Values are means ± SEM for 3-4. Significantly different from control, * p < 0.05, ** p < 0.01.

The concentration of muscle glycogen remaining after 30 min of swimming with and without administration of CAP is given in Fig. 6. The muscle glycogen concentration before swimming was not significantly different among the CAP and vehicle groups (vehicles 2.51 ± 0.36 vs. CAP 2.84 ± 0.32 mg/g muscle), suggesting that CAP administration did not increase consumption of muscle glycogen. After 30 min of swimming, the amount of muscle glycogen remaining was significantly greater in the mice administered CAP than in those given vehicle.

Effects of CAP on use of muscle glycogen during swimming
The concentration of muscle glycogen remaining after

effect of CAP on serum free fatty acids and glucose concentrations
To confirm that CAP-induced adrenaline secretion brought about an increase of fatty acid metabolism, the concentration of serum free fatty acids were measured at 30 min intervals after oral-administration of CAP (10 mg/kg). Serum free fatty acid concentrations gradually increased by CAP administration, reaching maximum within 2 hours before slowly decreasing as shown in Fig. 4. Serum glucose concentration increased immediately after CAP administration and then decreased within 60 min (Fig. 5).

Effects of adrenaline and CAP on adrenalectomized mice
To demonstrate that the adrenal was directly involved in the increase of swimming endurance capacity through adrenaline secretion, adrenalectomized mice were used. In intact mice that ingested CAP, the endurance increased by 50% compared with the vehicle administered intact mice (Fig. 7). However, swimming endurance in adrenalectomized mice was not significantly increased by CAP. When, instead of oral CAP administration, adrenaline was injected...
described previously, briefly, a water current is generated by circulating water with a pump in a pool.\(^{16}\) We used the apparatus because of the many advantages it offers in the evaluation of the endurance capacity of mice. Namely, the data show higher reproducibility than those obtained for treadmill running and forced swimming with a weight attached to the tail.\(^{16}\)

The flow rate of 7 liter/min, corresponds to a surface-current in the center of the pool of about 19 cm/s. The intensity of exercise, whereby the mice swim against the current, is estimated to be about 50% of VO\(_2\) \(_{\text{max}}\) from blood \(t\)-lactic acid concentration.\(^{16}\) To find whether the effect of CAP is influenced by the intensity of exercise, we have also examined the swimming capacity at 6 and 8 liter/min flow rates. Difference in swimming time between CAP and control mice were trends for a longer time at 6 liter/min than that of 7 liter/min. However we have used 7 liter/min in this study because the average time at 6 liter/min increased over a 120 min period and data of maximal swimming time were somewhat dispersed.

Hot red peppers, of which CAP is the major pungent component, have long been used as a seasoning, preservative, and medicine. CAP has striking pharmacological effects, especially on the primary afferent neurons.

In a previous study, Kawada \textit{et al.}\(^{19}\) have shown that CAP at a physiological level fed together with a high fat diet lowers the perirenal adipose tissue weight and serum triglyceride concentration in rats due to an increase in energy metabolism.\(^{19}\) Furthermore, the increase of energy metabolism has been proved to occur via increased secretion of catecholamine from the adrenal medulla.\(^{15}\) The increase of physiological catecholamine secretion by CAP occurred mainly through activation of the central nervous system, because up to \(8 \times 10^{-5}\) M CAP did not directly increase catecholamine secretion in a retrograde perfusion system of the left adrenal gland.\(^{13}\) In this study, oral administration of CAP (10 mg/kg) produced a marked increase in serum adrenaline concentrations within 30 min in resting mice. A second increment followed at 120 min. These bi-phasic increase in serum adrenaline concentration is consistent with the bi-phasic activation of adrenal sympath efferent nerve by intravenous administration of CAP.\(^{13}\)

The promotion of fat use by CAP had been demonstrated by measurement of the respiratory quotient (R.Q.). Kawada \textit{et al.}\(^{4}\) have demonstrated that the R.Q. was about 0.80 at rest when the rats were fed a high fat diet. It began to increase immediately after the administration of CAP, maximizing (0.86–0.92) within 30 min, the gradually decreasing to the initial level (about 0.80) at 60 min, before decreasing further to the minimum level (about 0.75) at about 120–150 min. The R.Q. level had returned to the initial level by 180 min. The decrease in R.Q. indicated the use of fatty acid as a fuel by CAP between 90 to 150 min, although the mechanisms of such a characteristic change in fuel use, \textit{i.e.}, from glucose to fat is unclear. Costill \textit{et al.}\(^{43}\) showed that elevated plasma free fatty acids resulted in decreased muscle glycogen use during exercise, and Ivy \textit{et al.}\(^{20}\) demonstrated that increased mobilization of fat increased endurance.

The sparing of muscle glycogen in CAP-administered mice, as shown in Fig. 6, further supported the metabolic increasing effect of CAP on endurance. Apparently, the reduced muscle glycogen usage was due to the increased

---

**Fig. 6.** Effects of CAP on Use of Muscle Glycogen during Swimming.

The remaining muscle (gastrocnemius) glycogen concentration after 30 min of swimming was measured on mice orally administered CAP (10 mg/kg) or vehicle solution 2 h before swimming. The data on the two groups were analyzed by an unpaired \(t\) test. Values are mean \(\pm\) SEM for 4–6. *Significantly different from control, \(p<0.01\).

---

**Fig. 7.** Effects of Adrenaline and CAP on Swimming Capacity of Adrenalectomized Mice.

The swimming capacity of adx mice (\(N=6\)) were measured after CAP (10 mg/kg) or adrenaline (0.3 mg/kg, i.p.). CAP or vehicle were orally administered 2 h and adrenaline were injected 30 min before swimming in a current of 7 liter/min. The endurance capacity of the three groups of adx mice (vehicle, CAP and adrenaline) was measured by a cross-experiment and the data were analyzed by the paired \(t\) test. Also, the swimming endurance of two groups of normal mice (\(N=24\), CAP and vehicle) were measured under the same conditions. The mean swimming time of intact and adx control mice were 40.0 \(\pm\) 2.2 min (intact) and 30.6 \(\pm\) 6.0 min (adx) (means \(\pm\) SEM). Values are means \(\pm\) SEM. Significantly different from adx mice that ingested CAP, * \(p<0.01\) (vs. adx mice ingested CAP).

i.p. 30 min before the test, endurance was increased by 58%. This suggests that the increase of swimming endurance capacity by CAP was due to the CAP-induced adrenaline increase.

**Discussion**

In this study, the swimming endurance capacity in mice was evaluated by using an adjustable-current swimming pool, which is a new forced-swimming apparatus for measuring maximum swimming time. Details have been
fatty acid use. Randle et al.\textsuperscript{21} reported the molecular mechanisms regulating fuel selection in muscle and have found that when presented, free fatty acids are used with a concomitant decrease in glucose uptake and use. Rennie et al.\textsuperscript{22} further reported that elevating the plasma concentration of free fatty acids decreased the rate of glycogen depletion during exercise. However, as shown in Fig. 4, a significant increase in endurance swimming capacity was not observed 3 hours after CAP administration in spite of a high plasma fatty acid concentration at 3 hours. This discrepancy is unclear, but it is not likely that an increase in plasma fatty acid concentration and an increase of fatty acid oxidation is always the same. In addition, less increase in plasma free fatty acids after CAP administration to conscious mice was observed in our preliminary experiment and these results are consistent with that of Graham et al.\textsuperscript{21} Further investigation about the plasma fatty acid oxidation is required instead of the plasma fatty acid concentration measurement we used.

The consumption of liver glycogen in preference to fat mobilization is an unavoidable process. However, the depletion of liver glycogen before swimming may not have affected endurance performance. The case of fasting before exercise may be applicable to the interpretation of this phenomenon. Dohm et al.\textsuperscript{23} indicated that fasting before exercise increases fat use and lowers the rate of muscle glycogen depletion. Fasting for 24 hours completely depletes liver glycogen but had only a small effect on muscle glycogen and under such conditions the rats ran for nearly 5 hours. It suggested that the mobilization and use of fat is more important than the liver glycogen store.

Orally administered CAP induced a bi-phasic increase in serum adrenaline concentration. The first phase was accompanied by an increase in serum glucose concentration. Although the concentration of serum free fatty acids may increase in both phases, free fatty acid use may not be active in the first phase because of the high glucose concentration in serum. A previous study by Kawada et al.\textsuperscript{24} has shown that the high respiratory quotient (R.Q.) within 60 min after CAP administration supports the idea of dominant use of glucose during this phase.

On the other hand, 2 hours after CAP administration, the serum glucose concentration was not high (Fig. 5), but the serum free fatty acid concentration increased markedly (Fig. 4). The respiratory quotient was low in this phase, suggesting that enough free fatty acids were recruited without an excessive increase in serum glucose. This may be advantageous to endurance activity in mice.

In adrenalectomized mice in the swimming test 30 min after injection of adrenaline, performance was improved. This is inconsistent with the increased endurance of the intact mice 2 hours after administration of CAP. However, adrenalectomized mice showed a markedly lower basal serum glucose level than intact mice,\textsuperscript{24} which is due to a lack of corticosterone. The change in blood glucose concentration was reported to be lower in adrenalectomized than in intact rats when adrenaline was injected intravenously.\textsuperscript{25} Therefore, even within such a short period of time, the serum free fatty acid could be used as a fuel, resulting in an increase of swimming endurance similar to that 2 hours after CAP administration.

From all these facts, it is likely that only those mice that started swimming 120 min after CAP administration were able to take advantage of the mobilization and use of fat to improve endurance.

The explanation that the adrenaline induced by CAP increased endurance, conflicts with previous studies that demonstrated that adrenaline increases muscle glycogenolysis at rest\textsuperscript{26,27,10} and during aerobic exercise,\textsuperscript{7–9} which is in general disadvantageous in endurance exercise. The conflict may be mainly due to the intervals between adrenaline injection and performance, as already discussed. Namely, at 2 hours after administration of CAP, the amount of glycogen spared through fat utilization is superior to that loss via glycogenolysis. This is consistent with the report by Chesley et al.\textsuperscript{21} that muscle glycogenolysis was unaffected by a doubling of the adrenaline concentration during intense aerobic exercise, and adrenaline induced by caffeine ingestion played a role in reducing muscle glycogen consumption during aerobic exercise.

We cannot rule out the possibility that unknown factors concomitant with endogenous adrenaline release are involved in the increase of endurance capacity. Graham et al.\textsuperscript{21} have demonstrated that a high dose of caffeine (9 mg/kg) elevated the plasma adrenaline concentrations at rest and during exercise and observed better endurance in well-trained runners. In the experiment, they reported lack of a significant increase in plasma free fatty acids. Therefore, they concluded that the effects of high doses of caffeine were real but were unclear as to whether the increase endurance performance was due to increased fat use and reduction of muscle glycogen consumption.

However, it is not likely that unknown predominant effects of CAP other than stimulation of the adrenal gland are responsible for the promotion of endurance capacity in mice, because as shown in Fig. 7, CAP did not increase endurance capacity when administered to adrenalectomized mice. Exogenous adrenaline given to adrenalectomized mice apparently reproduced the characteristic increase of endurance performance. This indicates that the adrenal gland was involved in the promotion of endurance capacity by CAP. However, these results cannot completely rule out the possibility that other physiologically active materials secreted from the adrenal gland by CAP ingestion increase the endurance capacity in mice, although such material remains unknown.

References