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EFFECTS OF A *VESPA* AMINO ACID MIXTURE IDENTICAL TO HORNET LARVAL SALIVA ON THE BLOOD BIOCHEMICAL INDICES OF RUNNING RATS

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ABSTRACT

A unique amino acid mixture identical in composition to that of larval saliva of the hornet, referred to as a *Vespa* amino acid mixture (VAAM) was prepared; the effect of VAAM on the blood biochemical indices and plasma amino acid composition of running rats was studied. Thirty min before running exercise, VAAM, a reference amino acid mixture with a composition identical to that of bovine casein (casein amino acid mixture, CAAM), or distilled water was orally administered to the rats. The blood glucose value in the animals given VAAM did not fall after 45 min of running, the value being significantly higher than that in the rats given CAAM or water ($P<0.05$). There was no significant change in the blood lactate value for those rats given VAAM, whereas the value in the rats given CAAM or water had significantly increased after 45 min of running ($P<0.05$). The plasma NEFA value for the VAAM group was significantly higher than that for the CAAM group after 45 min of running ($P<0.05$). Thirty min after the administration of VAAM or CAAM, the increase in the plasma concentration of amino acids reflected the composition of the amino acid mixture. However, after 45 min of running, the concentration of total plasma amino acid in rats given VAAM was maintained at a higher level than that in the rats given CAAM (3.57±0.30 vs 2.96±0.14 μM/l); the plasma concentrations of Gly, Thr, Tyr and Pro in the rats given VAAM were significantly higher than those in the rats given CAAM. These findings suggest that some amino acids in ingested VAAM were used for gluconeogenesis or as energy sources for muscles during exercise. In addition, a promotive effect of VAAM on the utilization of body fat as an energy source could have been involved in the changes of blood biochemical indices.

Key words: Hornet larval saliva, Amino acid, Blood glucose, Exercise, Plasma free fatty acid

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INTRODUCTION

The hornet (*Vespa*) is a social insect populating a big colony that nests in large combs. This large insect, with a length of 40 - 50mm, is an exterminator of the insect food chain and preys on many kinds of insects to feed the meat-eating hornet larvae. Adult hornets hunt by flying at a speed of over 30km/hr all day long, resulting in a daily flight distance of about 100km. Meat balls made by the hornet workers from the insect prey are given to their larvae in exchange for larval saliva. Since the adult hornets consume only liquid food, it must help to provide the energy and nutrients for flight and hunting.

A previous study has shown that the larval saliva of the hornet consisted mainly of free amino acids, and that the composition was similar among the five hornet species found in Japan (1). We subsequently prepared an amino acid mixture identical to the composition of the larval saliva, referred to as the *Vespa* amino acid mixture (VAAM), and examined its nutritional effect by an endurance exercise with swimming mice (2). The maximum swimming time of mice orally given the VAAM solution was significantly longer than that of control mice given another amino acid mixture. In order to elucidate the nutritional mechanism, we now report a study on the effect of VAAM on the blood biochemical indices and amino acid composition of running rats.

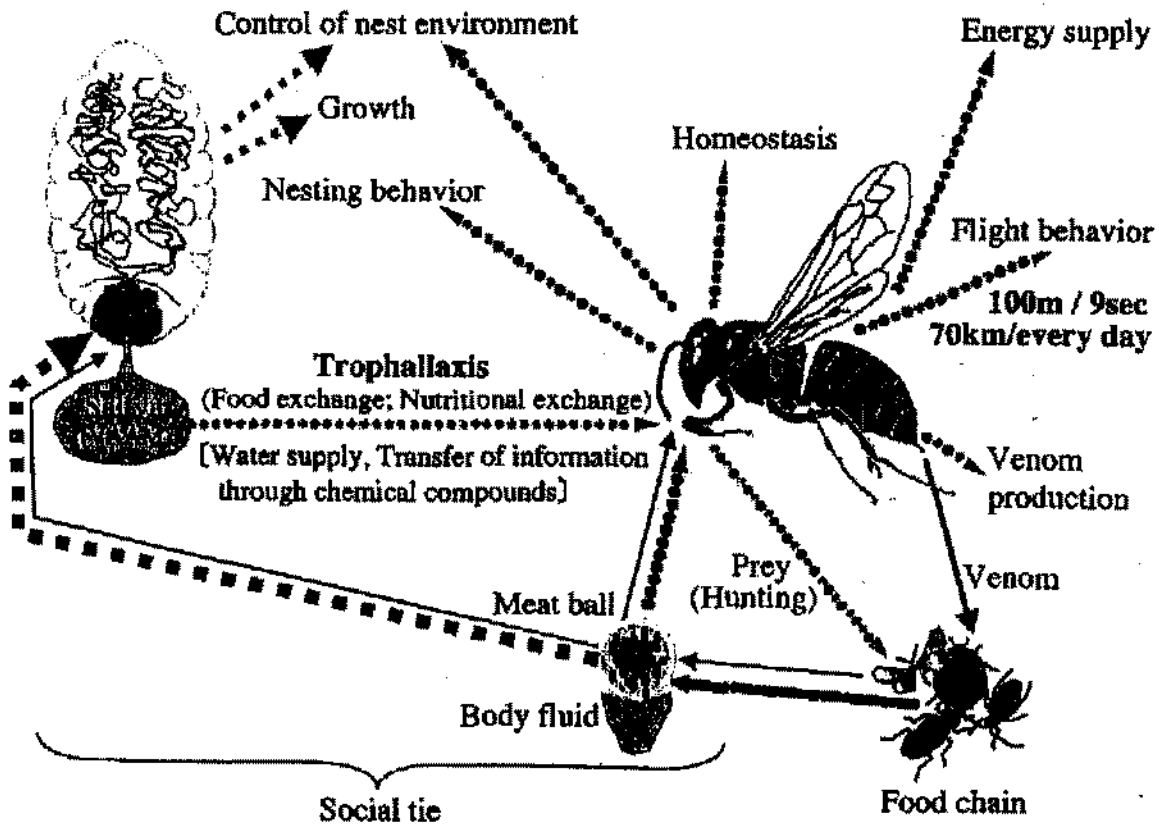
MATERIALS AND METHODS

Amino acid mixtures. An amino acid mixture identical to that in the larval saliva of *Vespa mandarinia* (1) was prepared (the *Vespa* amino acid mixture, VAAM). Another amino acid mixture identical to that in bovine casein (3) was prepared as a reference (the casein amino acid mixture, CAAM). The amino acid compositions of VAAM and CAAM are shown in Table 1. Each mixture was dissolved in distilled water (3.8g/100g) before being given to the animals.

Animals. Seventy-eight 4-wk-old male Sprague Dawley rats (Japan SLC Inc., Shizuoka, Japan) were housed in individual aluminum cages in a temperature-controlled (23±2°C) room with 50±10% humidity and a 12-h light-dark cycle. The care of the rats in this study conformed with *Guide for the Care and Use of Laboratory Animals* (4). The animals were given free access to a stock diet CE-7 (Clea Japan Inc., Tokyo, Japan) and tap water. They were run for 5-10 min/day on a motor-driven rodent treadmill (Natsume Seisakusho Co., Ltd., Tokyo, Japan) with an incline of 7° so that they were well-accustomed to being handled and to running on the treadmill. During this training period, the running speed was gradually increased until the rats were able to run at the rate of 20m/min.

At the age of 6 wk, a chronic catheter was implanted into the right jugular vein of each animal while anesthetized with sodium pentobarbital (40 mg/kg body weight; Dainippon Pharmaceutical Co., Ltd., Osaka, Japan). The surgical technique was based on the method described by Remie et al. (5), using a PE50 polyethylene cannula (Nippon Becton Dickinson Co., Ltd., Tokyo, Japan) and an anchor button (Instech Laboratories, Inc., Plymouth Meeting, PA, U.S.A.). Four days after the operation, the rats were run on the treadmill for 5 min/day at 20 m/min up a 7° incline for 3 days. The running training, and experiments 1 and 2 were carried out from 13:00 to 16:00.

Experiment 1. After being fasted overnight, twenty-four trained rats were assigned to three groups of eight animals each having a similar mean body weight (190g). One group of rats was orally given 0.4ml of VAAM, another was orally given 0.4ml of CAAM, and the third group was given distilled water as a control. Thirty minutes after the oral administration, the rats were subjected



to a running exercise at 23 m/min for 90 min. Blood samples were taken just before the administration of the test liquid (-30min), at the start (0min), halfway through (45min) and at the end (90min) of running to determine lactic acid, glucose and non-esterified fatty acid (NEFA). Another three groups of six trained rats were assigned to sedentary groups, who were treated with the same procedure as that just described, except for the running exercise.

Experiment 2. Six groups of six trained rats were assigned to the same program as that described for experiment 1, three running exercise groups and three sedentary groups. Blood samples were taken with the same regimen from the rats to determine the plasma free amino acid.

Analysis. Plasma glucose and non-esterified fatty acid (NEFA) were determined colorimetrically by using a measuring kit (Wako Pure Chemical Ind., Osaka, Japan), while plasma lactic acid was also determined by using a measuring kit (Sigma Diagnostics, St. Louis, MO. U.S.A.). Plasma free amino acids were determined by a model 835 amino acid analyzer (Hitachi.

Tokyo, Japan) after removing protein by adding a 3% sulfosalicylic acid solution.

Statistics. The data reported in FIGs.1 to 3 are expressed as means±SEM, and in FIGs. 4 and 5, as means. One-way ANOVA with a subsequent Bonferroni test was used at each time point to test for any significant differences in the mean values of the experimental groups ($P<0.05$). Repeated measures ANOVA with a subsequent Bonferroni test was done to evaluate changes within a group over time ($P<0.05$). The analysis was performed by using StatView version 4.0 statistical software (Abacus Concepts, Inc., Berkeley, CA, U.S.A.).

RESULTS

Biochemical indices. After having been administered with one of the test solutions, the blood glucose value in the rats given CAAM or water increased slightly, and then decreased significantly after 45 and 90 min of running exercise (FIG. 1a). In contrast, the blood glucose value in the animals given VAAM did not fall after 45 min of running, the value being significantly higher than that in each of the other two groups ($P<0.05$). In the sedentary groups, the blood glucose values did not change significantly ($P<0.05$) during the test (FIG. 1b).

The blood lactate value in the rats given CAAM or water significantly increased after 45 min of running ($P<0.05$) and then decreased at the end of the running exercise (FIG. 2a). In contrast, there was no significant change in the value for those rats given VAAM during the test. In the sedentary groups, the blood lactate value did not change significantly ($P<0.05$) during the test (FIG. 2b).

The plasma NEFA value was high in all the animals just before the oral administration because of overnight fasting (FIG. 3a and FIG. 3b). Plasma NEFA in the rats given VAAM or CAAM decreased at 0 min, and then increased significantly ($P<0.05$) while running. The value for the VAAM group was significantly higher than that for the CAAM group after 45 min of running ($P<0.05$). The plasma NEFA value for the sedentary rats given VAAM was higher than that for the sedentary rats given CAAM 75 min after the administration, although the difference was not significant.

Plasma amino acids. There was no significant difference in the individual plasma amino acid concentrations among the experimental groups before being given a test liquid (FIG. 4a and FIG. 5a). Thirty min after being administered with one of the amino acid solutions, the plasma amino acid concentrations had increased significantly ($P<0.05$), except that glutamine (Glu), methionine (Met) and cysteine (Cys) did not increase in the VAAM group, and Cys and glycine (Gly) did not increase in the CAAM group (FIG. 4b). The increases in the plasma amino acid concentrations of the sedentary rats given VAAM or CAAM was similar to those of the running rats 30 min after the administration (FIG. 5b).

After 45min of running, the individual amino acid concentrations had decreased in all the groups, except for tyrosine (Tyr) in the VAAM group (FIG. 4c). The concentrations of Gly, alanine (Ala), valine (Val), threonine (Thr), Tyr, lysine (Lys) and proline (Pro) in the rats given VAAM were significantly higher than those in the rats given distilled water ($P<0.05$). In addition, the concentrations of Gly, Thr, Tyr and Pro in the rats given VAAM were significantly higher than the equivalent values in the rats given CAAM ($P<0.05$ for Gly, Tyr and Pro; $P<0.1$ for Thr). In the

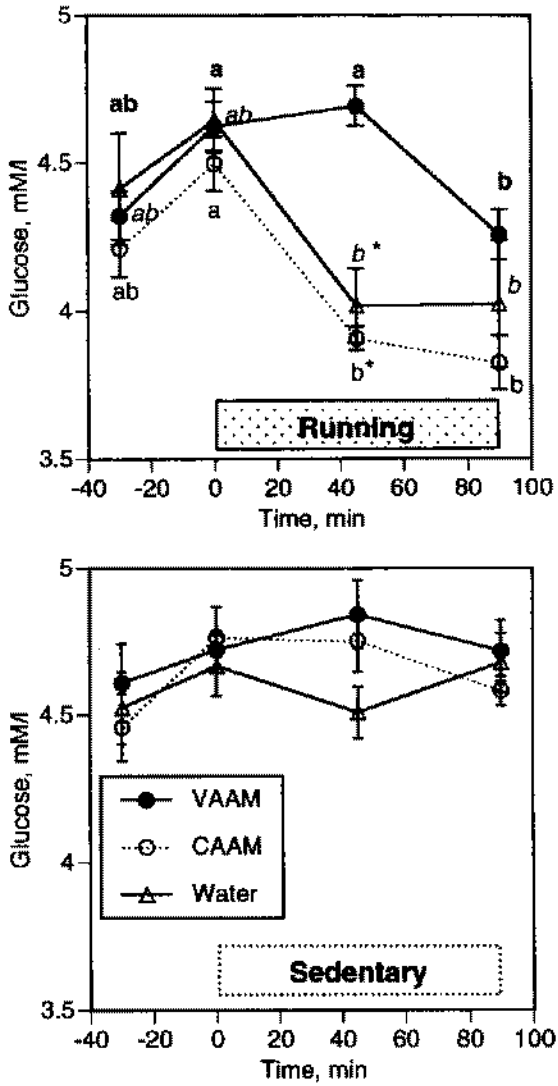


FIG. 1. Changes with time of the effect of administering to rats a vespa amino acid mixture (VAAM), casein amino acid mixture (CAAM) or distilled water (Water) on blood glucose. Thirty minutes after the administration, rats were assigned to running exercise (top) or kept sedentary (bottom). Values are means with their standard errors represented by vertical bars. At each time point, * indicates a significant difference compared with the value for rats given VAAM ($P<0.05$). Within a group, time points not sharing a common letter are significantly different ($P<0.05$).

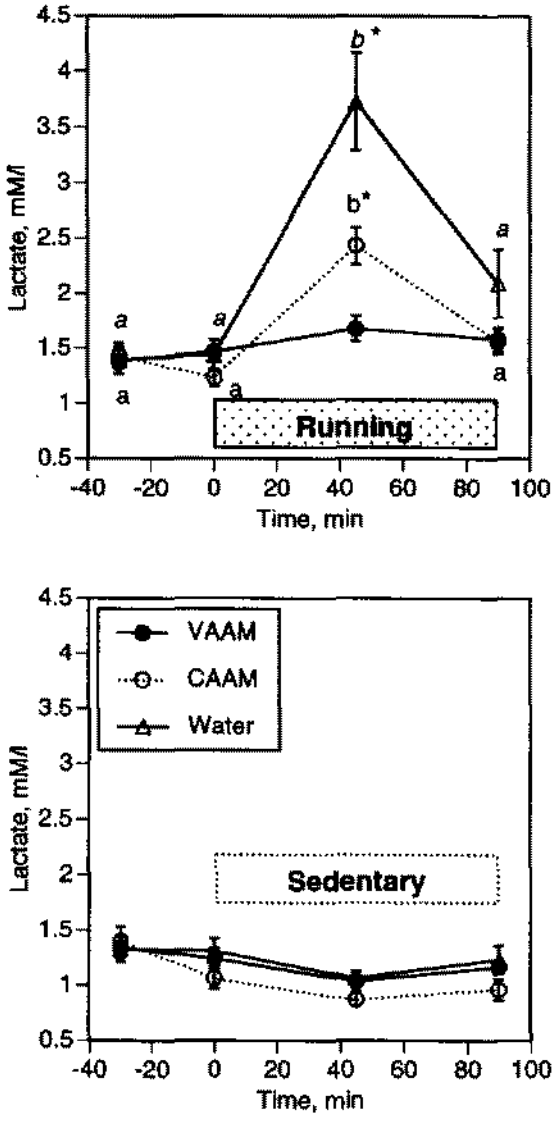


FIG. 2. Changes with time of the effect of administering to rats a vespa amino acid mixture (VAAM), casein amino acid mixture (CAAM) or distilled water (Water) on blood lactate. Thirty minutes after the administration, rats were assigned to running exercise (top) or kept sedentary (bottom). Values are means with their standard errors represented by vertical bars. At each time point, * indicates a significant difference compared with the value for rats given VAAM ($P<0.05$). Within a group, time points not sharing a common letter are significantly different ($P<0.05$).

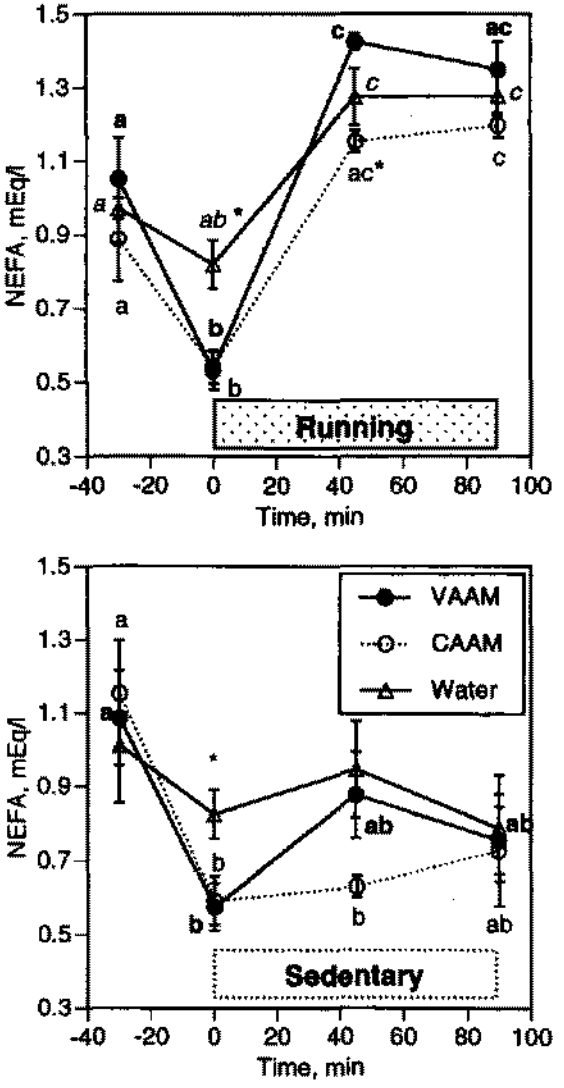


FIG. 3. Changes with time of the effect of administering to rats a vespa amino acid mixture (VAAM), casein amino acid mixture (CAAM) or distilled water (Water) on plasma non-esterified fatty acid (NEFA). Thirty minutes after the administration, rats were assigned to running exercise (top) or kept sedentary (bottom). Values are means with their standard errors represented by vertical bars. At each time point, * indicates a significant difference compared with the value for rats given VAAM ($P<0.05$). Within a group, time points not sharing a common letter are significantly different ($P<0.05$).

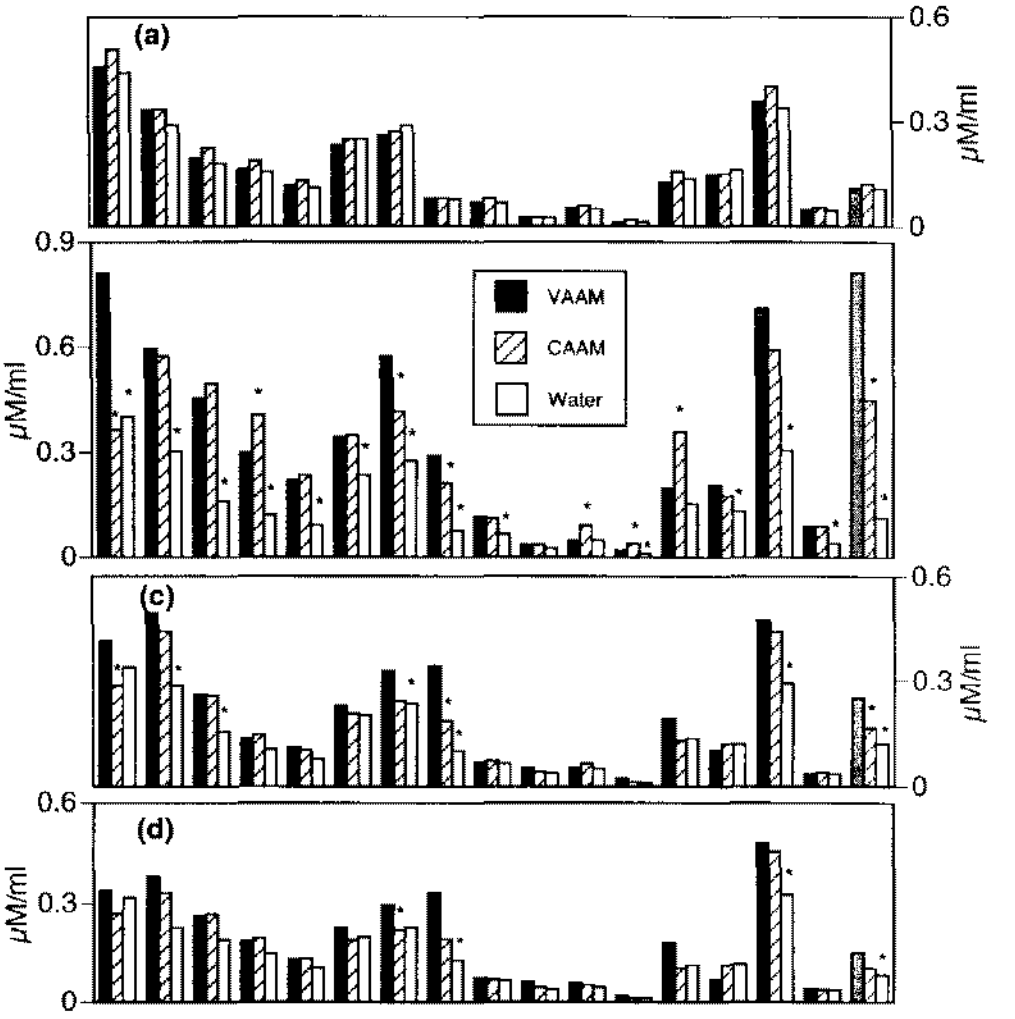


FIG. 4. Effect of administering to rats a vespa amino acid mixture (VAAM), casein amino acid mixture (CAAM) or distilled water (Water) on the plasma amino acid concentration during running exercise. The composition of plasma amino acids is presented (a) before the administration, (b) 30 min after the administration, (c) 45 min after running and (d) 90 min after running. Values are expressed as means. As for individual amino acids, * indicates a significant difference compared with the value for rats given VAAM at each time point ($P<0.05$).

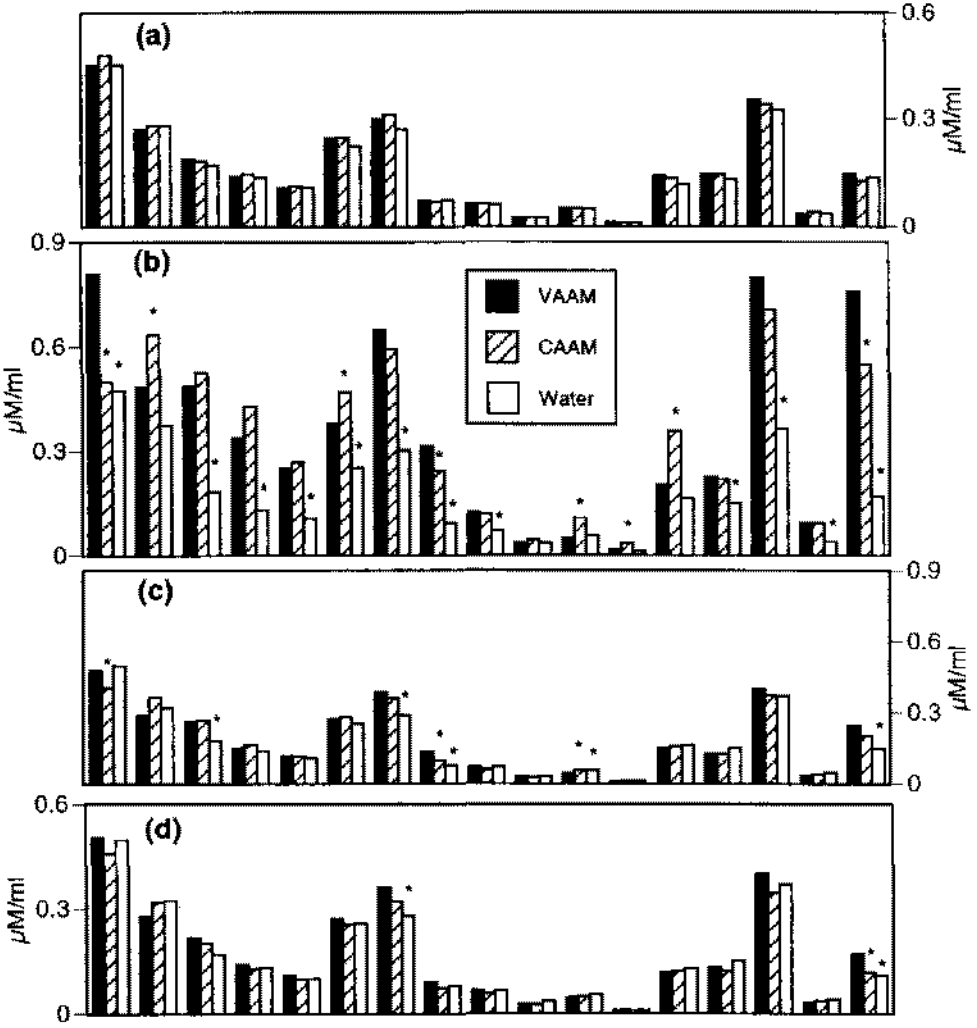


FIG. 5. Effect of administering to rats a vespa amino acid mixture (VAAM), casein amino acid mixture (CAAM) or distilled water (Water) on the plasma amino acid concentration in sedentary rats. The composition of plasma amino acids is presented (a) before the administration, and (b) 30 min, (c) 75 min and (d) 120 min after the administration. Values are expressed as means. As for individual amino acids, * indicates a significant difference compared with the value for rats given VAAM at each time point ($P<0.05$).

CAAM group, the concentrations of Val and Lys were significantly higher than those in the control group, but none of the values was higher than those in the VAAM group. When the animals were allowed to be sedentary, no increase in the concentration of Ala or Lys in the rats given VAAM was apparent 75 min after the administration (FIG. 5c). The concentration of Tyr in the sedentary rats given VAAM was lower than that in the running rats given the same solution, although the value was significantly higher than that in the sedentary rats given CAAM or water.

At the end of running, the concentrations of Val, leucine (Leu), serine (Ser), Thr, Tyr and Lys were little decreased in the rats given VAAM, in comparison to the equivalent values after 45min of running (FIG. 4d). The concentrations of Thr, Tyr, Lys and Pro in the rats given VAAM were significantly higher than those in the rats given distilled water after 90min of running ($P<0.05$ for Tyr, Lys and Pro; $P<0.1$ for Thr). When the animals were allowed to be sedentary, the concentrations of Thr and Pro in the rats given VAAM were significantly higher than those in the running rats given water 120 min after the administration.

DISCUSSION

The results for blood glucose and lactate in the running rats given VAAM were comparable to those in mice after swimming to exhaustion (2). The higher blood glucose value in the rats given VAAM after 45 min of running indicates less consumption of glucose, or an increase in gluconeogenesis or glycogenolysis. The gluconeogenic process plays a major role in glucose homeostasis during exercise (6). Although amino acids cannot supply much energy compared with glucose and fatty acids during exercise, the gluconeogenic process is a potentially important pathway for the utilization of amino acids. There was little difference between VAAM and CAAM in the amount of gluconeogenic amino acids whose carbon chains can be transformed to phosphoenolpyruvate and thus to glucose. The rate of gluconeogenesis from Ala is much higher than that of the other amino acids in the liver (7,8). The plasma Ala concentrations in the rats given VAAM were not significantly higher than those in the rats given CAAM 30 min after the administration, and after 45 and 90 min of running. Therefore, the higher blood glucose value for the rats given VAAM after 45 min of running could not be simply explained by the amount of gluconeogenic amino acids in the mixture.

Blood lactate concentration represents a balanced state between lactate production by glycolysis in active muscles and its removal by decomposition or gluconeogenesis in other organs such as the liver. Therefore, the lower blood lactate value in the rats given VAAM after 45 min of running indicates a possible decrease in glycolysis. Alternatively, the administration of VAAM might have promoted the gluconeogenesis from lactate through activation of the Cori cycle. This possibility remains to be examined.

The plasma NEFA value was higher not only in the running rats but also in the sedentary rats given VAAM. The increase in plasma NEFA suggests an increased oxidation of fatty acids and inhibition of carbohydrate utilization (9). Insulin infusions have resulted in a decrease of plasma NEFA in trained rats (10) and an inferior running performance (11). Therefore, it is hypothesized that the administration of VAAM could have promoted lipolysis or inhibited the insulin action. If an administration of VAAM can promote lipolysis, it could cause a glucose sparing effect which is suggested from the data on blood glucose, promotion of the expenditure of fat as an energy substrate should be confirmed by a further study measuring the respiratory quotient and biochemical indices

related to lipid metabolism.

Changes in the plasma amino acid levels following exercise have been reported in humans (12,13) and animals (14,15). Dohm et al. (14) have reported a decrease in the concentration of plasma acidic amino acids and Ala, and an increase in branched-chain amino acid (BCAA), Tyr, Lys, Met and Phe in trained rats after a bout of running exercise to exhaustion. In contrast, Ji et al. (15) have reported a decrease in the concentration of plasma Ala, aspartic acid (Asp), asparagine (Asn), arginine (Arg), His, Lys, Met, phenylalanine (Phe), Pro, Ser, Thr and BCAA, and an increase in Glu in running rats. In the present study, the changes in plasma amino acids concentrations of the rats given water are not fully consistent with the data just described: the plasma concentration of Gly, Ser, Thr and Arg decreased, and that of Tyr increased after running exercise. This inconsistency might reflect individual differences in the plasma amino acid composition of the animals used due to their diet or other experimental conditions (16).

Thirty min after the administration of VAAM or CAAM, increases in the plasma concentration of amino acids reflected the composition of the amino acid mixture. However, after 45 min of running, the concentration of total plasma amino acids in the rats given VAAM was maintained at a higher level than that in the rats given CAAM (3.57±0.30 vs 2.96±0.14 μM/l); the plasma concentrations of Gly, Thr, Tyr and Pro in the rats given VAAM were significantly higher than those in the rats given CAAM. Since a net breakdown of body protein would be the source of amino acids for increased oxidation and gluconeogenesis during exercise (17), the relatively higher concentrations of plasma amino acids during exercise by the administration of VAAM could have contributed to the amino acid source or inhibited proteolysis in the liver (18) and other organs including muscles. Under catabolic conditions such as fasting and exercise, BCAA may serve as important energy sources for muscles (8). The concentration of BCAA in VAAM was lower than that in CAAM, the plasma concentration of BCAA in the rats given VAAM also being lower than that in the rats given CAAM 30 min after the administration, although it was higher than that in the rats given water. Therefore, it seems unlikely that BCAA played a major role in the energy sources for muscles during exercise in the rats given VAAM.

The whole-body Tyr concentration was reportedly increased (14), and the plasma BCAA /aromatic amino acids ratio (Fischer ratio) was depressed (19) in rats after exhaustive exercise. The decrease in the Fischer ratio was high in the rats given VAAM, but it is not likely that the degree of fatigue was higher in this group, because the previous study (2) and the results of the blood glucose and lactate values suggested less fatigue. An increase in the plasma concentration of Tyr indicates a net breakdown of protein during exercise (17). In this study, the plasma concentration of Tyr was increased with the time of running, the values in the rats given VAAM being much higher among the groups. However, the higher value for plasma Tyr concentration in the rats given VAAM reflects the high Tyr concentration in the amino acid mixture, the increase during exercise being relatively small and similar among the groups. On the other hand, the increase in plasma Tyr concentration, a metabolic precursor of norepinephrine, may be related to central fatigue or the metabolic state of energy via the hormonal pathway. Lehnert et al. (20) have reported that animals receiving a Tyr-enriched diet displayed neither a stress-induced depletion of norepinephrine nor behavioral depression. In addition, pretreatment with supplemental Tyr prevented the behavioral depression and hypothalamic norepinephrine depletion that had been observed after acute stress (21). The serum levels of catecholamines were reportedly enhanced during exercise (11), and an interaction of catecholamines with α_2-adrenoceptors resulted in a lipolytic action in rats (22). If the serum levels of

catecholamines in the rats given VAAM could be proved to have been higher than those in the other groups, the foregoing hypothesis that the administration of VAAM could promote lipolysis and the utilization of body fat as an energy source might be supported. Tyr and Phe have been reported to affect the synthesis of catecholamines, and certain amino acids serve as a neurotransmitter (23,24). The administration of VAAM may have had an effect on amino acids in the brain, but this remains to be clarified.

The administration of VAAM prior to running revealed an inhibitory action on the depression of the blood glucose level, lower blood lactate level and high NEFA value during exercise. Although these results cannot be fully explained by the changes in plasma amino acid concentrations, the findings suggest that some amino acids in ingested VAAM were used for gluconeogenesis or for energy sources in muscles during exercise. In addition, a promoting effect of VAAM on the utilization of body fat as an energy source could have been involved in the changes of blood biochemical indices. Further studies are necessary to clarify the effect of VAAM on lipid metabolism via hormonal action during resting as well as during exercise.

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