Branched-Chain Amino Acids in Exercise

Exercise-Dependent Preference for a Mixture of Branched-Chain Amino Acids and Homeostatic Control of Brain Serotonin in Exercising Rats

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ABSTRACT In this article we discuss studies showing that rats are able to regulate their intake of BCAA depending on the level of exercise, and that they will choose a solution of BCAA over water during times of intense exercise. We found that the voluntary intake of a solution made of BCAA, L-arginine and L-glutamine positively correlated with the timing and volume of exercise during the dark (active) period of the circadian rhythm. In the second behavioral protocol, in which rats were fed BCAA fortified diet (210%, wt:wt), we observed voluntarily increased volume of physical activity beginning from d 4 of feeding on. In the second, neuro-behavioral, part of the study we measured the brain content of 5-hydroxytryptamine (5-HT) as well as plasma amino acid profiles in well-trained exercising rats to test a hypothesis that BCAA may alleviate central aspects of fatigue. A solution made of BCAA, L-arginine, and L-glutamine applied before running elevated the BCAA/trypophan plasma ratio at the end of and after running, and decreased 5-HT release in the lateral hypothalamus and amygdala after running, when compared with the controls. The exercise-related shift in the fluid preference toward a BCAA-based solution suggests an ergogenic benefit. The forced-running study shows the lateral hypothalamus and possibly amygdala might be the critical brain regions implied in the central effects of a BCAA-based solution. J. Nutr. 136: 548S–552S, 2006.

KEY WORDS: • branched-chain amino acids • exercise • hypothalamus • amygdala • rat

Central nervous system aspects of physical performance are a subject of continuing discussion. Part of this discussion focuses on the plasma amino acids, specifically on the BCAAs, tryptophan (TRP), and the ratio of that portion of TRP in blood not bound to albumin to the sum of the concentrations of the BCAAs (this ratio is an indicator of TRP uptake into brain) (1). A reduction in this ratio seems to augment physical performance (2–4). The free TRP/BCAA ratio (ratio of plasma free TRP concentration to the sum of the concentrations of the BCAAs) rises during exercise (2), because BCAA levels decline due to increased peripheral oxidation, and free TRP levels rise, because fatty acid mobilization displaces TRP from albumin (5,6). This is associated with a rise in brain TRP and an increase in neuronal serotonin (5-HT) synthesis and release. The bases for these effects in brain are: 1) TRP and the BCAA compete for a common transport carrier into brain, and the rate of TRP uptake reflects the concentrations in blood of TRP relative to those of its transport competitors; 2) brain TRP concentrations respond to changes in the competitive uptake of TRP into brain; and 3) the rate of 5-HT synthesis and release follows brain TRP concentrations (7). As noted above, sustained exercise is associated with increased brain 5-HT activity (8,9). Because 5-HT neurons are involved in brain circuits that control arousal, sleepiness, and mood, it has been hypothesized that during exercise, the activation of 5-HT neurons produces central fatigue and thereby diminishes physical performance (2,10). In this context, BCAA ingestion has been viewed as a possible way to reduce brain 5-HT during exercise, and thus central fatigue. Data supporting this BCAA hypothesis include the observation that BCAA supplements can increase the time to fatigue in both humans (10) and rats (11,12). However, not all studies have found such improvements (13–15).

The effects of BCAA supplements reportedly depend on several factors, such as the use of BCAAs with carbohydrates (2), the mode of BCAA application (16), the nutritional status and physical aptitude of the subjects (11), and the length and intensity of exercise. The argument has been made that the positive effects of the BCAA are greater during exercise in the heat, or in a competitive race, where the central component of fatigue (i.e., central fatigue) plays a significant role (2). Correspondingly, central fatigue is an important factor not only in exercise, but also in the chronic fatigue syndrome and in postoperative recovery. Chronic fatigue syndrome patients cannot adjust the circulating ratio of TRP/BCAA during and
after exercise (17), and postoperative elderly patients are characterized by increased plasma-free TRP (18).

We have been interested in testing the BCAA hypothesis and over the past several years have conducted behavioral and neurochemical studies in rats under controlled laboratory conditions. Our behavioral approach has differed from that taken by others, who have studied BCAA effects on fatigue in exercising rats (e.g., 12, 16). We have examined the influence of exercise on the rat’s selection of freely available solutions of amino acids, a selection model frequently used in our laboratory (19, 20) based on the idea that, with time, rats might sense some benefit of ingesting some amino acids over others in an exercising paradigm. Our neurochemical approach involves the direct examination of neuronal 5-HT release by exercising rats. In particular, we have evaluated 5-HT release in several brain regions in exercising and nonexercising rats. We wondered if some of the discrepancies in observing CNS effects of BCAA might be due to the choice of recording site. Because of the possibility that part of the BCAA effects may be related to emotional aspects of exercise (2), we speculated that 5-HT release during exercise would be specifically increased only in those brain areas involved in either recognition of peripheral amino acids (e.g., hypothalamus) or emotions (e.g., hypothalamus, amygdala).

The solution we tested in these studies contained BCAAs, L-arginine, and L-glutamine, as it reflects a popular formulation in common use (21).

Behavioral studies

In one behavioral paradigm, we examined running activity in adult male rats in cages containing running wheels (Habitest L91–16S, Coulbourn Instruments). The test group was fed a standard laboratory diet fortified with BCAAs (20 g/kg); the control group was fed the standard laboratory diet containing glutamine instead of the BCAAs (20 g/kg). All rats had free access to food and water. Running behavior was measured in 10-min intervals. We observed that rats consuming the control diet ran approximately the same distance each d. However, the animals consuming the BCAA-supplemented diet gradually increased their running each d, such that by d 4, their daily activity was significantly greater than that of their control counterparts (Fig. 1).

In a second study (21), adult male rats were adapted to the same cages (containing running wheels), but the cages were each equipped with a pellet dispenser and 2 fluid dippers. One fluid diper provided water; the other supplied a BCAA-based solution (15.2 mmol/L l-leucine, 9.9 mmol/L l-isoleucine, 11.1 mmol/L l-valine, 16.6 mmol/L l-glutamine, 13.9 mmol/L l-arginine). Rats could freely choose the solutions. Eating, drinking, and running activity were recorded in 30-s intervals. The relation between running activity and the volume of each fluid ingested during the dark period (1900–0700) on d 3 of the study (each data point is a single rat) is presented in Figure 2, panel A. We observed that rats running >1500 m during the dark period consumed significantly more of the BCAA-based solution than water (ANOVA; F = 70.86, P = 0.0002). The relationship between running distance and fluid intake for each solution (water, BCAA solution) was best fit by the 3-parametric Hill function (r = 0.86 for BCAA-based solution and r = 0.78 for water). Food intake was unrelated to running distance (Fig. 2, panel B). We then examined the temporal relationship of fluid intake to running activity during the dark period (Fig. 2, panel C), and observed that the peak in nocturnal running activity corresponded to the highest intake of the BCAA-based solution.

Together, these results (Figs. 1 and 2) show that: 1) rats constrained to consume chronically elevated amounts of BCAA in their standard diet gradually increase their running activity over rats ingesting a standard diet containing a control amino acid (glutamine); and 2) rats given a standard diet, water, and water containing BCAAs, show a positive relationship between nocturnal running distance and volume of the BCAA solution consumed (and a negative correlation with volume of water ingested); and 3) the nocturnal period of greatest running is temporally associated with the time of greatest intake of the BCAA solution.

Although the present data do not give an answer on the innate versus learned character of the above observations, the results of the first study suggest that a 3–4-d learning period may have been involved (Fig. 1). But overall, the results suggest that exercising rats have the ability, when given the choice, to increase their intake of BCAAs in times of elevated physical activity, and perhaps vice-versa to adjust physical activity to match the BCAA content of their diet. One may speculate that when the physical stress of exercise reaches a certain level of homeostatic balance, the adaptive needs for specific amino acids (BCAAs) increase, leading to an enhancement of their intake.

Neuro-behavioral study: homeostatic control of the brain 5-HT

We next examined whether neuronal 5-HT release during exercise would be specifically increased in those brain areas involved in recognition of amino acids (hypothalamus) and emotional response to physical stress (amygdala), and if BCAA ingestion would moderate such an effect (21). Rats were implanted with guide cannulas (20) and subsequently trained to run daily on a treadmill for 1-h periods. The microdialysis probe was then inserted through the guide cannula during a treadmill session to measure 5HT release. At intervals, small blood samples were taken to measure amino acids and corticosterone. Animals were studied at treadmill speeds of 5 and 25 m/min; the treatments were an 8 mL oral infusion of water or a BCAA-containing solution (15.2 mmol/L l-leucine, 9.9 mmol/L l-isoleucine, 11.1 mmol/L l-valine, 16.6 mmol/L l-glutamine, 13.9 mmol/L l-arginine). Treadmill running at 25 m/min increased circulating corticosterone concentrations,
but the rise was the same, whether water or the amino acid solution was infused. The amino acid solution raised the plasma levels of the BCAA, and produced a small decline in TRP, such that the ratio of BCAA/TRP increased (or the ratio of TRP/BCAA decreased) (Fig. 3). This change would be expected to reduce TRP uptake into brain (7).

In rats infused with water, neuronal 5-HT release in the lateral hypothalamus was significantly elevated by the end of the 1-h run at the higher speed tested (25 m/min) (Fig. 4). This

**FIGURE 2** Free nocturnal behaviors of 15 male Wistar rats. The relation between running distance during the dark period is compared with fluid intake (panel A) and diet intake (panel B) in each individual rat. A 3-parametric Hill function was used for data regression ($r = 0.86$ for BCAA-based solution, and $r = 0.78$ for water). *Different from water intake in rats running >1500 m/dark period ($P < 0.05$, ANOVA followed by t test). Time-dependent activities during dark period were compared in panel C. Individual data (running distance and fluid intakes) from all rats were smoothed by Sigma-Plot 5.0 low pass recursive filter. This filter reduced components above the half-power point, generating the filtered output $f(x)$ from the data $x(t)$: $f(x) = A \times f(x - 1) + (1 - A) \times x(t)$, where time, $x(t)$ is original data, and $A$ is computed from the specific half-power point obtained via a series of regressions. Values are means of 15 rats. To facilitate comparison, running activity is expressed as gray area. A peak in drinking activity was identified as a time-point, when drinking increased ≥200% over 60 min (identified by a number in parentheses). Running gradually increased during the dark period and the highest running activity was between 0400 and 0500 (identified by an asterisk). The peak in running activity corresponded well to the highest peak in the BCAA-based solution intake. Reprinted with permission from Smriga et al. (21).

**FIGURE 3** Panel A, plasma BCAAs; Panel B, tryptophan (TRP); and Panel C, BCAA/TRP ratio in rats trained in a treadmill for 10 d. Blood was sampled directly from heart in ether-anesthetized rats. Rats were placed into the treadmill for 55 min (acclimatization). Thereafter, they were infused orally with 8 mL water or 8 mL of the BCAA-based solution. Running (25 m/min) started 5 min after the infusion and lasted for 1 h. The data shown by the columns denoted as immediately before running were obtained 5 min after the infusion. Values are means ± SEM of 3 rats. *Different from water group, $P < 0.05$ (ANOVA followed by t test); #Different from prerunning values in the same group ($P < 0.05$, 1-way ANOVA followed by Duncan’s multiple range test (MRT)). Reprinted with permission from Smriga et al. (21).
increase was considerably blunted in rats infused with the BCAA-containing solution. A similar, but less-marked effect of the amino acid solution was observed in the central area of amygdala (Fig. 4). Serotonin terminals projecting to the lateral hypothalamus and the amygdala participate in the regulation of stress, food intake, and fatigue. Thus, the suppression of the amino acid solution showed no difference in food intake as a function of the amount of this solution they consumed (Fig. 2, panels A and B). Third, the observed changes in neuronal 5-HT release could have directly ameliorated central fatigue, as suggested by other research (2).

Conclusion

In rats, ingestion of a BCAA-containing solution appears to have a positive action on physical activity. At least part of this effect may be related to effects on neuronal 5-HT release in the brain, and thus 5-HT-dependent processes in brain areas relating to central fatigue. These effects may contribute to the alleviation of central fatigue in exercising subjects. Branched-chain amino acids also positively influence the metabolic response of muscle to exercise (24). Effects on brain thus represent only a portion of the overall actions of BCAA on physical performance.

LITERATURE CITED


