Central Nervous System Effects of Caffeine
and Adenosine on Fatigue

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CNS Caffeine Delays Fatigue

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ABSTRACT

Caffeine ingestion can delay fatigue during exercise, but the mechanisms remain elusive. This study was designed to test the hypothesis that blockade of CNS adenosine receptors may explain the beneficial effect of caffeine on fatigue. Initial experiments were done to confirm an effect of CNS caffeine and/or the adenosine A$_1$/A$_2$ receptor agonist, NECA, on spontaneous locomotor activity. Thirty min prior to measurement of spontaneous activity or treadmill running, male rats received Caffeine, NECA, Caffeine-plus-NECA, or Vehicle during 4 sessions separated by approximately 1 week. CNS Caffeine and NECA (i.c.v.) were associated with increased and decreased spontaneous activity, respectively, but Caffeine-plus-NECA did not block the reduction induced by NECA. CNS Caffeine also increased run time to fatigue by 60% and NECA reduced it by 68% versus Vehicle. However, unlike the effects on spontaneous activity, pretreatment with Caffeine was effective in blocking the decrease in run time by NECA. No differences were found following peripheral (i.p.) drug administration. Results suggest that caffeine can delay fatigue through CNS mechanisms, at least in part by blocking adenosine receptors.

KEYWORDS: Ergogenic aids, treadmill running, spontaneous activity, endurance capacity, rats
INTRODUCTION

Ingestion of caffeine has been shown to delay fatigue during prolonged intense exercise in both human and animal models (7, 8, 21, 22, 23, 27, 28, 36), although there are conflicting reports on its effects (1, 12, 37, 39). With ingestion of 3-9 mg/kg of caffeine, exercise time to fatigue during intensive running or cycling is typically increased by 20%-50% (8, 21, 22, 36, 38). However, the mechanism remains elusive. Costill and associates (8, 11) suggested that caffeine increased the availability of free fatty acids (FFA), producing greater fat metabolism in the active muscle and inhibiting carbohydrate metabolism, thus sparing muscle glycogen. However, the evidence for increased fat metabolism is mixed, with some investigators finding this effect (5, 30, 38) while others have not (2, 23, 39). In addition, there is now strong evidence that the ergogenic effect of caffeine is not due to muscle glycogen sparing (1, 2, 5, 20, 27, 30). Furthermore, increased plasma epinephrine, which is suggested to be the stimulus for the increase in plasma FFA, is not always associated with enhanced endurance performance (23, 28). In fact, epinephrine may be counterproductive to endurance performance due to its stimulatory effects on glycogen breakdown and the resulting increase in blood and muscle lactate (2, 27, 28, 30). Therefore, these mechanisms are now thought not to play a primary role in the fatigue delaying effects of caffeine (19).

Another possibility that has received little scientific attention is that the ergogenic effect of caffeine ingestion occurs primarily through stimulation of the central nervous system (CNS). Caffeine, a potent adenosine antagonist, is a CNS stimulant that easily crosses the blood-brain-barrier (BBB) due to its lipophilic properties (32). It has been shown to counteract most of the inhibitory effects of adenosine on neuroexcitability (14, 18),
neurotransmitter release (34), arousal (35), and spontaneous activity (3). Although caffeine can alter CNS function by inhibiting phosphodiesterase (PDE) activity, blocking GABA_A receptors, and mobilizing intracellular calcium (15), the doses required are 20, 40, and 500 times higher, respectively, than that required to block adenosine receptors (14, 15). Therefore, blockade of adenosine receptors is now believed to underlie most of the CNS effects of caffeine under normal physiological circumstances (14, 15, 34). Blocking CNS adenosine receptors may also help to explain the fatigue-delaying properties of caffeine, although no such studies have been reported in the literature.

Adenosine is a normal cellular constituent that is regulated mainly by ATP metabolism and other adenine nucleotides (29). Adenosine concentrations increase in muscle and plasma during muscular contraction. Adenosine concentrations also increase progressively in the brain during wakefulness and then decrease during sleep (26, 35). Physiologically, adenosine plays an important role in regulation of blood flow and as an inhibitory modulator of neuronal excitability and synaptic transmission of the brain via activation of adenosine receptors (10, 29). Adenosine inhibits the release of most brain excitatory neurotransmitters (14, 24, 34), especially dopamine (DA)(33). Behaviorally, these changes are associated with reduced arousal, increased sleep (26, 35), and suppression of spontaneous behavioral activity (3, 25).

The primary purpose of this study was to determine the effects of intracerebroventricular (i.c.v.) injection of caffeine and the adenosine A1 and A2 receptor agonist 5’-N-ethylcarboxamidoadenosine (NECA) on treadmill run time to fatigue in rats. NECA was chosen for this study since caffeine is a non-selective adenosine receptor antagonist and it is not known which of the four subtypes of adenosine receptors may be
involved in an effect of caffeine on fatigue. However, A2b and A3 receptors are relatively less active than A1 and A2a receptors under normal physiological conditions (14). We hypothesize that CNS administration of caffeine will increase run time to fatigue, whereas NECA will reduce run time to fatigue. Furthermore, pretreatment with caffeine prior to NECA will attenuate the fatigue inducing effects of NECA.

**MATERIALS AND METHODS**

*Animals*

Male Wistar rats (Harlan Sprague Dawley, IN) were used in this study, with initial age of 5 weeks and body weight of 200-250 grams. The rats were randomly assigned to i.c.v. or intraperitoneal (i.p.) injection groups. The animals were allowed to adapt to the vivarium for 2 days in individual polypropylene cages in a room maintained at 22°C and a constant light-dark cycle (light 7:00 a.m. to 7:00 p.m.). The rats were given food and water ad libitum. The study was approved by the Institutional University Animal Use Committee and follows the American Physiological Society Guiding Principles in the Care and Use of Animals.

*Procedure of Treadmill Acclimation and Cannula Implantation:*

Rats were given two weeks of treadmill acclimation, (specially designed animal treadmill by Columbus Instruments, OH, model Exer-4) during which they ran for 15 minutes per day. The treadmill speed was slowly increased from 8 m • min⁻¹, 7.5% grade at the beginning, progressing to 20 m • min⁻¹ at the end of the acclimation period. Gentle hand prodding and mild electric shock (20 mV, 1.67 Hz) were combined to encourage the animals to run throughout the study.

After the first two weeks of acclimation, rats assigned to the i.c.v. group were anesthetized with sodium pentobarbital (50mg/kg, i.p.) and cannulae were implanted
bilaterally into the lateral ventricles. Brain coordinates were AP -0.6 mm, L +/-1.5 mm, and DV -4.5 mm with respect to bregma and dura according to Paxinos and Watson’s atlas. Proper placement of the cannula were confirmed by i.c.v. injections of 1 µL of 1% angiotensin II, which resulted in water consumption of no less than 5 mL over a period of 30 minutes.

After 7 days of recovery from surgery, the rats were again acclimated to treadmill running for another one to two weeks, until they were able to run easily for at least 15 minutes per day for 5 consecutive days at a speed of 20 m • min⁻¹, 7.5% grade. Animals that were unable to run for 20 minutes at speed of 20 m • min⁻¹, 7.5% grade were excluded.

Drug Preparation and Administration

Four drug treatments were used in the study: NECA, caffeine, caffeine-plus-NECA, and vehicle. NECA and caffeine were obtained from Research Biochemicals Internationals (RBI, Natick, MA) and the vehicle solution (Normosol-R) was obtained from Abbott Laboratories, IL. The vehicle solution has been used as a control solution in other studies involving i.c.v. infusions of drugs and tissue microdialysis (4). The vehicle solution consisted of 90.0 mM sodium chloride, 27.0 mM sodium acetate, 23.0 mM sodium gluconate, 5.0 mM potassium chloride, and 1.5 mM magnesium chloride hexahydrate. NECA was dissolved first with 0.1 N HCl and then diluted with vehicle solution, while caffeine was dissolved directly with vehicle solution. All the drug solutions were freshly prepared and the pH was adjusted to 7.4 prior to injection into the animals. Each rat was injected with 0.1 µg of NECA and/or 200 µg of caffeine 20 min prior to behavioral testing. The caffeine-plus-NECA treatment included the same caffeine and NECA doses as were used individually. These dosages were based on extrapolation from other studies that have
used these same drugs in behavioral tests, but via other routes of administration (3, 17).
During i.c.v. injections, 5 µL of drug solutions were slowly administered into each side of the lateral ventricle over 2.5 minutes, after which another 1.5 minutes was allowed for drug diffusion.

**Spontaneous Locomotor Activity**

Spontaneous locomotor activity was done on a small group of animals prior to the treadmill exercise trials to insure that the chosen drug dosages were effective in producing general behavioral stimulation/inhibition. No data were available in the literature regarding the dose-response of these drugs when administered i.c.v. Spontaneous locomotor activity was tested using an open field box (76 cm x 76 cm, divided by 8 lines into 25 squares). Rats were randomly given CNS injection with one of the four drug treatments: NECA (n=4), Caffeine (n=4), Caffeine-plus-NECA (n=4), or Vehicle (n=3). Fifteen minutes after the injection, the rats were placed into the open field box and the behavioral activity was video-taped for ten minutes. Spontaneous locomotor activity was measured as “lines crossed” (horizontal movement crossing the floor lines) and “rears” (raising the frontal paws vertically). The number of lines crossed and rears were measured in 5-minute blocks for a total of 10 minutes.

**Treadmill Run Time to Fatigue:**

In the CNS groups (n=10), each rat was injected (i.c.v.) with one of the four drugs (NECA, Caffeine, Caffeine-plus-NECA, or Vehicle) in one testing session. The other drugs were then given successive testing sessions at approximately 1-week intervals to allow full recovery from the exercise bout and wash-out of the drugs. On two days during the 4-7 day recovery period, all rats were exercised for 15-minutes (20 m · min⁻¹, 7.5% grade) to
maintain acclimation to the treadmill protocol. All rats received all four drug treatments in a randomized and counter-balanced design to minimize possible order effects. In the Peripheral groups (n=8), the rats were injected (i.p.) with the same doses of the drugs given centrally in the same repeated-measure, counter-balanced fashion. During each testing session, the drugs were administered 20 minutes prior to treadmill exercise.

The treadmill speed assigned to individual rats was determined by a treadmill test that was done prior the experimental treatment. Rats that ran longer than 2.5 hours at a speed of 20 m min⁻¹, 7.5% grade were assigned to 23 m min⁻¹, 7.5% grade protocol. The others were assigned to 20 m min⁻¹, 7.5% grade protocol. By allowing this small variation in treadmill speed, overall run time to fatigue was much more consistent at approximately 90 minutes.

During treadmill running, rats were encouraged to run by combined gentle hand prodding and mild electric shock (20 mV, 1.67 Hz). Rats received electric shock when they rested on the electric wires that were installed at the end of each treadmill lane. Volitional fatigue was determined as the time at which the rats would no longer run on the treadmill and simply choose to rest on the electric wires despite continual hand prodding and mild electric shocks for 30 seconds.

**Statistical Procedures**

For the test of treadmill run time to fatigue, one-way ANOVA with repeated measures was used to detect any differences in mean run time to fatigue among the drug treatments. Post-Hoc procedures were used to detect specific differences in mean run time to fatigue between the vehicle treatment and each of the drug treatments (Vehicle vs. NECA, Vehicle vs. Caffeine, and Vehicle vs. Caffeine-plus-NECA) in both the CNS injection groups
and Peripheral injection groups. For the tests of spontaneous locomotor activity, one-way ANOVA was used to detect any differences in mean values among the experimental treatments. Student's t-tests were used to detect specific differences in the mean values between vehicle treatment and each drug treatment. The Bonferroni method was used to protect the overall significance level of 0.05 when there were multiple t-test comparisons.

**RESULTS**

The data on spontaneous locomotor activity (Fig. 1 and Fig. 2) indicate that the drug dosages chosen for i.c.v. administration in this experiment were effective in producing general behavioral stimulation and inhibition. This was consistent with the effects of these drugs when given at different doses via other routes of administration. During the 10-minute open field test, both measures of spontaneous locomotor activity (lines crossed and rears) were significantly lower in the rats treated with the NECA treatment than with the Vehicle treatment (p=0.001 and p=0.021, respectively). The Caffeine treatment was associated with greater rears (p=0.040), but there was only a trend (probably due to the small sample size) toward higher lines-crossed activity (p=0.079). Caffeine-plus-NECA did not block the reduction of spontaneous locomotor activity induced by NECA. The same pattern was also observed in each of the 5-minute testing blocks.

Mean treadmill run time to fatigue was significantly different among the four CNS drug treatments (Fig. 3). Treadmill run time was 76.46 ± 8.98 min (mean ± SE) with the Vehicle. Caffeine increased run time by 60% (119.05 ± 12.28 min, p=0.004), whereas it was reduced by 68% in NECA (24.25 ± 6.99 min, p=0.001). When Caffeine was administered 5 minutes prior to NECA injection (Caffeine-plus-NECA), it blocked the reduction in run time to fatigue that occurred in NECA and was not different than Vehicle (63.62 ± 14.98 min,
p=0.397). In contrast, when peripheral injections of the same drugs were administered (n=8), run time was similar in all treatments (88.63 ± 11.16 min, 90.5 ± 9.83 min, 102.25 ± 15.21 min, and 85.30 ± 14.63 min for the Vehicle, Caffeine, NECA and Caffeine-plus-NECA treatments, respectively, p=0.329, see Fig. 4).

DISCUSSION

Caffeine has been used in athletic competitions primarily due to its ergogenic effects. The effective dosage of caffeine, without apparent adverse effects, ranges from 3-9 mg/kg. At this dose, caffeine can increase exercise time to fatigue by 20%-50% in humans during intensive running and cycling (7, 8, 21, 22, 23, 27, 28) and in rats during prolonged treadmill running (31, 36). However, ergogenic benefit can be influenced by the dose of caffeine, type and intensity of exercise, previous caffeine use, training status, and individual variation (19). Costill and colleagues postulated that caffeine improves endurance exercise by sparing muscle glycogen through increased fat oxidation, which resulted from increased epinephrine concentrations (8, 11). However, previous studies have found that caffeine ingestion does not always spare muscle glycogen (1, 2, 5, 6, 20, 27, 30), increase plasma FFA (2, 19, 23, 39), or reduce the respiratory exchange ratio during exercise (7, 20, 23). In addition, elevated plasma epinephrine, which often but not always increases plasma FFA during exercise, is not necessarily associated with enhanced endurance exercise performance (23, 28). In fact, increased epinephrine may be counterproductive to endurance capacity if it results in increased glycogenolysis as indicated by increased blood and muscle lactate in some studies (2, 27, 28, 30).

The ergogenic effects of caffeine may also involve mechanisms within the CNS. Its lipophilic nature enables it to easily cross cell membranes including the BBB (32). Ingestion
of caffeine may therefore act through stimulation of the CNS to improve exercise performance. But to our knowledge, no study in the literature has addressed possible direct CNS effects of caffeine on fatigue during prolonged exercise. Our study shows that CNS administration of caffeine at a dose of 200 µg per rat (about 0.6 mg/kg), which is much less than the effective dose given peripherally (6 mg/kg) (36), does increase treadmill run time to fatigue in rats by approximately 60%. The same dose of caffeine given peripherally (i.p.) is ineffective.

Until recently, little was known about the potential CNS mechanisms that could explain a benefit of caffeine given centrally on endurance performance. We hypothesized that the blockade of adenosine receptors by caffeine seemed to be the most likely mechanism of CNS stimulation and delayed fatigue. This theory is based on the following observations. Adenosine is an endogenous inhibitory modulator for neuronal excitability and synapse transmission (10). Adenosine also inhibits the release of most brain excitatory neurotransmitters, particularly DA (13, 25, 34) and may reduce DA synthesis (33). Decreases in DA, along with an increases in 5-HT (generally associated with behavioral suppression) have been linked to central fatigue during exercise (9). In addition, adenosine has been shown to reduce arousal, induce sleep (26, 35), and suppress spontaneous activity (3, 25), which are all behaviors associated with increases in 5-HT (9).

Adenosine concentrations are mainly regulated by ATP metabolism. Increased breakdown of ATP induces an increase in adenosine concentration (29). With muscular contraction, adenosine concentrations are raised in the working muscle and in the blood. Brain adenosine has not been measured during exercise, but increases progressively during wakefulness and decreases during sleep (26, 35) and under various hypoxic/ischemic
conditions (29). Therefore, we hypothesized that blocking adenosine receptors with caffeine would reverse the inhibitory effects of adenosine, and thus delay fatigue. This may also attenuate the increase of the 5-HT/DA ratio in the brain that has been suggested to play a role in central fatigue (9, 15, 17, 31, 33, 34).

The results of this study support our hypothesis that CNS administration (i.c.v.) of the selective adenosine A1 and A2 receptor agonist NECA, significantly reduced run time to fatigue, whereas caffeine (i.c.v.) increased run time to fatigue. The inhibitory effects of NECA on run time to fatigue were also reversed by pretreatment with caffeine (i.c.v.), suggesting that the ergogenic effects of caffeine (i.c.v.) are mediated through blockade of the adenosine receptors.

The effects of CNS administration of caffeine and NECA on various peripheral metabolic markers of fatigue cannot be totally ruled out in this study. Although it is not likely that these drugs produced direct effects on peripheral metabolic function by crossing the BBB (27, 28) given the very small doses given centrally, the drugs, especially caffeine, may have had indirect effects on metabolism by altering sympathetic nervous and/or neuroendocrine activity. However, this was apparently not the case in a small subset of rats (n = 3-4 rats/drug group) that we examined following 30 minutes of treadmill running in each drug condition. No significant differences existed in muscle glycogen, plasma glucose, FFA, and corticosterone between the Caffeine, NECA, and Vehicle groups. There was a slightly lower liver glycogen content in the Caffeine group versus Vehicle. However, since liver glycogen depletion is an important cause of fatigue during prolonged exercise, delayed fatigue in the Caffeine groups despite a reduction in liver glycogen argues more favorably for a CNS (i.e., behavioral) mechanism of action. However, these observations, while helpful,
must be viewed with caution given the very small sample size, and in the case of muscle
glycogen, the method of sacrifice (i.e., decapitation was used for analysis of brain
neurotransmitters). However, these observations are generally supported by a series of
studies by Winder and co-workers (1, 2, 39) who demonstrated that intravenous infusions of
relatively large doses of caffeine (25 mg/kg) had no effect on muscle and liver
glycogenolysis, and little if any effect on plasma glucose, FFA, insulin, and glucagon
concentrations during 60-90 min of treadmill running in trained and untrained rats.

The inhibitory and stimulatory effects of CNS administration of NECA and caffeine
on spontaneous locomotor activity observed in this study are consistent with the findings in
previous studies (3, 17). Barraco and colleagues reported that i.c.v. injections of NECA at
doses of 0.032 µg to 0.1 µg per mouse produced a dose-related reduction on spontaneous
locomotor activity (3). Meanwhile, rats treated with caffeine (i.p.) at doses of 25-50 mg/kg
showed significantly greater locomotor activity than saline-treated rats (17). However,
unlike Barroco’s study in which pretreatment of caffeine (i.p.) at 30 mg/kg blocked the
locomotion-inhibitory effects of NECA (3), our study demonstrated that pretreatment of
caffeine (i.c.v.) at 0.6 mg/kg did not attenuate the inhibitory effects of NECA (i.c.v.) on
spontaneous locomotion. It is important to note that the previous study administered 30
mg/kg of caffeine peripherally (i.p.), while we used only 0.6 mg/kg centrally (i.c.v.). There
are no other reports using i.c.v. injections of caffeine to antagonize adenosine effects. It is
not certain whether different doses and routes of administration can explain this discrepancy.

It is also interesting that pretreatment with caffeine (i.c.v.) blocked the inhibitory
effects of NECA (i.c.v.) on run time to fatigue, but not on spontaneous locomotor activity.
The explanation for this difference is not clear. However, treadmill exercise and open field
behavioral activity involve different motivations. Treadmill exercise uses electric shock to
encourage running, whereas open field behavior is motivated by curiosity and anxiety and are
therefore likely to involve different neurochemical mechanisms. It is also possible that the
selected dose of caffeine was too low to antagonize the NECA effects on behavioral activity
in rats, though it was effective in treadmill exercise.

In conclusion, CNS administration of caffeine increased treadmill run time to fatigue
and spontaneous locomotor activity in rats. CNS administration of the adenosine receptor
agonist NECA inhibited treadmill run time to fatigue and spontaneous locomotor activity in
rats. Pretreatment with caffeine blocked the inhibitory effects of NECA on exercise
performance, though not on spontaneous behavioral activity. Peripheral administration (i.p.)
of the same drugs at the same doses had no effect on treadmill run time to fatigue. These
results indicate that caffeine can act specifically within the central nervous system to delay
fatigue, at least in part by blocking adenosine receptors. Since caffeine easily crosses the
blood brain barrier, these results would also suggest that the CNS also plays an important
role in the ergogenic effect of caffeine ingestion. The precise independent contribution of
caffeine at the central (behavioral) and peripheral (metabolic) levels awaits further research.
We would argue that some interaction at both levels is likely with the predominant effects
occurring centrally.
REFERENCES


Figure 1. Effect of i.c.v. injection of NECA (n=4), Vehicle (n=4), Caffeine (n=4), and Caffeine-plus-NECA (n=3) in male rats on lines crossed during an open field test. All values are means ± SE. The number of lines crossed for each group was 72 ± 36, 284 ± 77, 437 ± 51, and 67 ± 54, respectively.

* Denotes lines crossed significantly different compared to Vehicle (p<0.05).

Figure 2. Effect of i.c.v. injection of NECA (n=4), Vehicle (n=4), Caffeine (n=4), and Caffeine-plus-NECA (n=3) in male rats on rearing during an open field test. All values are means ± SE. The number of rears was 5 ± 5, 48 ± 9, 69 ± 7, and 5 ± 3, respectively.

* Denotes rears significantly different compared to Vehicle (p<0.05).

Figure 3. Effect of i.c.v. injection (n=10) of NECA, Vehicle, Caffeine, and Caffeine-plus-NECA on treadmill run time to fatigue in male rats. All values are means ± SE. The mean run times were 24.25 ± 6.99 min, 76.46 ± 8.98 min, 119.05 ± 12.28 min, and 63.62 ± 14.98 min, respectively.

* Denotes run time significantly different compared to Vehicle (p<0.05).

Figure 4. Effect of i.p. injection (n=8) of NECA, Vehicle, Caffeine, and Caffeine-plus-NECA on treadmill run time to fatigue. All values are means ± SE. The mean run times were 102.25 ± 15.21 min, 88.63 ± 11.16 min, 90.5 ± 9.83 min, and 85.30 ± 14.63 min, respectively.
NECA Vehicle Caffeine Caf+NECA

Running Time (minutes)

NECA     Vehicle     Caffeine     Caf+NECA

*