Central nervous system effects of caffeine and adenosine on fatigue

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Davis, J. Mark, Zuowei Zhao, Howard S. Stock, Kristen A. Mehl, James Buggy, and Gregory A. Hand. Central nervous system effects of caffeine and adenosine on fatigue. *Am J Physiol Regul Integr Comp Physiol* 284: R399–R404, 2003. First published October 24, 2002; 10.1152/ ajpregu.00386.2002.—Caffeine ingestion can delay fatigue during exercise, but the mechanisms remain elusive. This study was designed to test the hypothesis that blockade of central nervous system (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 5′-*N*-ethylcarboxamidoadenosine (NECA) on spontaneous locomotor activity. Thirty minutes before measurement of spontaneous activity or treadmill running, male rats received caffeine, NECA, caffeine plus NECA, or vehicle during four sessions separated by ~1 wk. CNS caffeine and NECA (intracerebroventricular) 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% vs. 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 after peripheral (intraperitoneal) drug administration. Results suggest that caffeine can delay fatigue through CNS mechanisms, at least in part by blocking adenosine receptors.

ergogenic aids; treadmill running; spontaneous activity; endurance capacity; rats

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, adeno-

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sine 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 injection of caffeine and the adenosine A_1 and A_2 receptor agonist 5'-N-ethylcarboxamidoadenosine (NECA) on treadmill run time to fatigue in rats. NECA was chosen for this study because caffeine is a nonselective 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 before 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 wk and body weight of 200–250 g. The rats were randomly assigned to intracerebroventricular or intraperitoneal 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 AM to 7:00 PM). 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's "Guiding Principles for Research Involving Animals and Human Beings."

Procedure of treadmill acclimation and cannula implantation. Rats were given 2 wk of treadmill acclimation (specially designed animal treadmill by Columbus Instruments, OH; model Exer-4) during which they ran for 15 min/day. The treadmill speed was slowly increased from 8 m/min, 7.5% grade at the beginning, progressing to 20 m/min 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 2 wk of acclimation, rats assigned to the intracerebroventricular group were anesthetized with pentobarbital sodium (50 mg/kg ip), and cannulas were implanted bilaterally into the lateral ventricles. Brain coordinates were anteroposterior −0.6 mm, lateral ±1.5 mm, and dorsoventral −4.5 mm with respect to bregma and dura according to Paxinos and Watson's atlas. Proper placement of the cannulas was confirmed by intracerebroventricular injections of 1 µl of 1% ANG II, which resulted in water consumption of no less than 5 ml over a period of 30 min.

After 7 days of recovery from surgery, the rats were again acclimated to treadmill running for another 1–2 wk, until they were able to run easily for at least 15 min/day for 5 consecutive days at a speed of 20 m/min, 7.5% grade. Animals that were unable to run for 20 min at speed of 20 m/min, 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 RBI (Natick, MA), and the vehicle solution (Normosol-R) was obtained from Abbott Laboratories. The vehicle solution has been used as a control solution in other studies involving intracerebroventricular infusions of drugs and tissue microdialysis (4). The vehicle solution consisted of (in mM) 90.0 sodium chloride, 27.0 sodium acetate, 23.0 sodium gluconate, 5.0 potassium chloride, and 1.5 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 before injection into the animals. Each rat was injected with 0.1 µg of NECA and/or 200 µg of caffeine 20 min before 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 intracerebroventricular injections, 5 µl of drug solutions were slowly administered into each side of the lateral ventricle over 2.5 min, after which another 1.5 min was allowed for drug diffusion.

Spontaneous locomotor activity. Spontaneous locomotor activity was done on a small group of animals before the treadmill exercise trials to ensure 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 intracerebroventricularly. Spontaneous locomotor activity was tested using an open field box (76 × 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 videotaped for 10 min. 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-min blocks for a total of 10 min.

Treadmill run time to fatigue. In the CNS groups ($n = 10$), each rat was injected intracerebroventricularly with one of the four drugs (NECA, caffeine, caffeine plus NECA, or vehicle) in one testing session. The other drugs were then given in successive testing sessions at ~1-wk intervals to allow full recovery from the exercise bout and washout of the drugs. On 2 days during the 4- to 7-day recovery period, all rats were exercised for 15 min (20 m/min, 7.5% grade) to maintain acclimation to the treadmill protocol. All rats received all four drug treatments in a randomized and counterbalanced design to minimize possible order effects. In the peripheral groups ($n = 8$), the rats were injected intraperitoneally with the same doses of the drugs given centrally in the same repeated-measure, counterbalanced fashion. During each testing session, the drugs were administered 20 min before treadmill exercise.

The treadmill speed assigned to individual rats was determined by a treadmill test that was done before the experimental treatment. Rats that ran >2.5 h at a speed of 20 m/min, 7.5% grade, were assigned to a 23 m/min, 7.5% grade protocol. The others were assigned to a 20 m/min, 7.5% grade protocol. By allowing this small variation in treadmill speed, overall run time to fatigue was much more consistent at ~90 min.

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 chose to rest on the electric wires despite continual hand prodding and mild electric shocks for 30 s.

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 (Figs. 1 and 2) indicate that the drug dosages chosen for intracerebroventricular 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-min 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
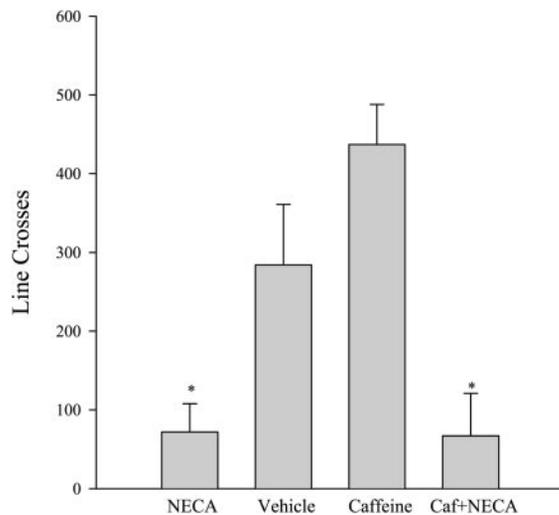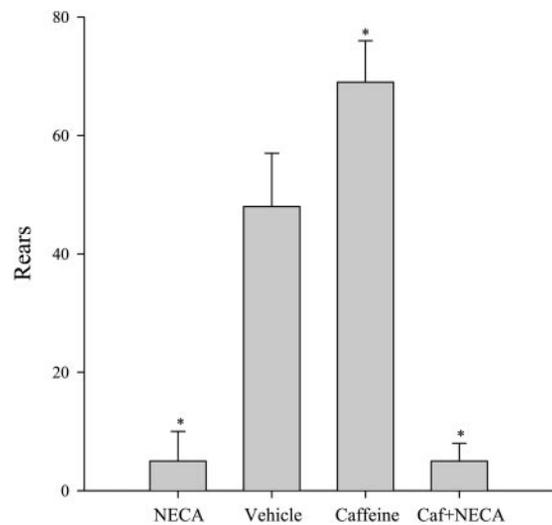
Fig. 2. Effect of intracerebroventricular injection of NECA ($n = 4$), vehicle ($n = 4$), caffeine ($n = 4$), and caffeine + NECA ($n = 3$) in male rats on rearing during an open field test. All values are means ± SE. The number of rears was 5 ± 5, 48 ± 9, 69 ± 7, and 5 ± 3, respectively. *Rears significantly different compared with vehicle ($P < 0.05$).

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-min 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 (means ± 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 = $

Fig. 1. Effect of intracerebroventricular injection of 5′-*N*-ethylcarboxamidoadenosine (NECA; $n = 4$), vehicle ($n = 4$), caffeine ($n = 4$), and caffeine (Caf) + 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. *Lines crossed significantly different compared with vehicle ($P < 0.05$).
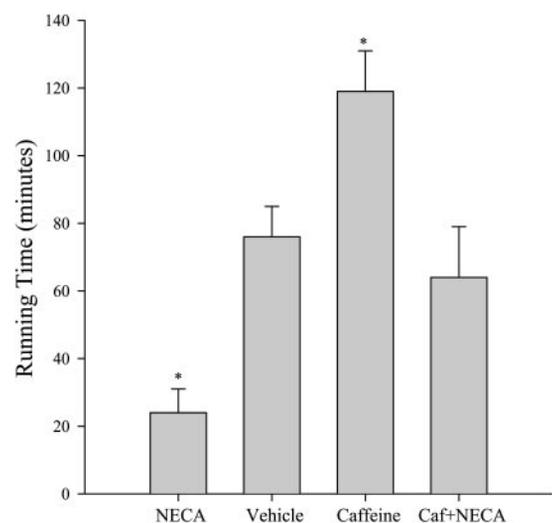
Fig. 3. Effect of intracerebroventricular injection ($n = 10$) of NECA, vehicle, caffeine, and caffeine + NECA on treadmill run time to fatigue in male rats. All values are means ± SE. The mean run times were 24.25 ± 6.99, 76.46 ± 8.98, 119.05 ± 12.28, and 63.62 ± 14.98 min, respectively. *Run time significantly different compared with vehicle ($P < 0.05$).

0.001). When caffeine was administered 5 min before NECA injection (caffeine plus NECA), it blocked the reduction in run time to fatigue that occurred in NECA and was not different from 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, 90.5 ± 9.83, 102.25 ± 15.21, 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 to 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 (8, 11) postulated that caffeine improves endurance exercise by sparing muscle glycogen through increased fat oxidation, which resulted from increased epinephrine concentrations. 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 increase 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).
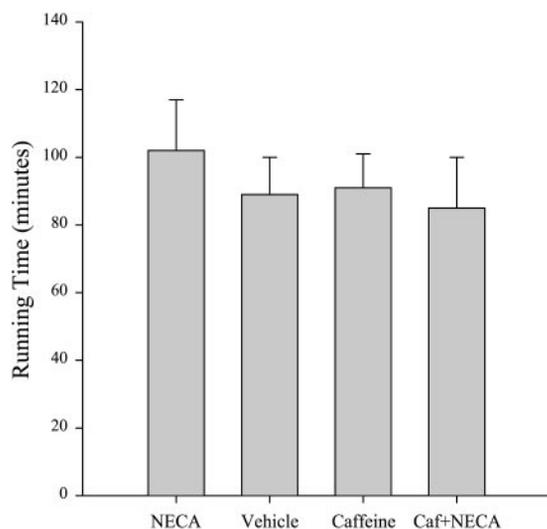

Fig. 4. Effect of intraperitoneal injection ($n = 8$) of NECA, vehicle, caffeine, and caffeine + NECA on treadmill run time to fatigue. All values are means ± SE. The mean run times were 102.25 ± 15.21, 88.63 ± 11.16, 90.5 ± 9.83, and 85.30 ± 14.63 min, respectively.

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. However, 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/rat (~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 ~60%. The same dose of caffeine given peripherally (intraperitoneally) 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). It also increases 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 intracerebroventricular CNS administration of the selective adenosine A_1 and A_2 receptor agonist NECA significantly reduced run time to fatigue, whereas intracerebroventricular caffeine increased run time to fatigue. The inhibitory effects of NECA on run time to fatigue were also reversed by intracerebroventricular pretreatment with caffeine, suggesting that the ergogenic effects of intracerebroventricular caffeine 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 after 30 min 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 vs. vehicle. However, because 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 death (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 (3) reported that intracerebroventricular injections of NECA at doses of 0.032–0.1 µg/mouse produced a dose-related reduction on spontaneous locomotor activity. Meanwhile, rats treated with caffeine intraperitoneally at doses of 25–50 mg/kg showed significantly greater locomotor activity than saline-treated rats (17). However, unlike Barroco's study in which intraperitoneal pretreatment of caffeine at 30 mg/kg blocked the locomotion-inhibitory effects of NECA (3), our study demonstrated that intracerebroventricular pretreatment of caffeine at 0.6 mg/kg did not attenuate the inhibitory effects of intracerebroventricular NECA on spontaneous locomotion. It is important to note that the previous study administered 30 mg/kg of caffeine peripherally (intraperitoneally), while we used only 0.6 mg/kg centrally (intracerebroventricularly). There are no other reports using intracerebroventricular 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 intracerebroventricular pretreatment with caffeine blocked the inhibitory effects of intracerebroventricular NECA 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, although 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, although not on spontaneous behavioral activity. Peripheral (intraperitoneal) administration 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 CNS to delay fatigue, at least in part by blocking adenosine receptors. Because caffeine easily crosses the BBB, 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.

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