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L-theanine partially counteracts caffeine-induced sleep disturbances in rats

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ABSTRACT

L-theanine has been reported to inhibit the excitatory effects of caffeine. The present study examined the effects of L-theanine on caffeine-induced sleep disturbances in rats. Rats received the following drug pairings: saline and saline (Control), 7.5 mg/kg caffeine and saline, or 7.5 mg/kg of caffeine followed by various doses of L-theanine (22.5, 37.5, 75, or 150 mg/kg). Vigilance states were divided into: wakefulness (W), transition to slow-wave sleep (tSWS), slow-wave sleep (SWS), and rapid-eye-movement sleep (REMS). Caffeine significantly increased the duration of W and decreased the duration of SWS and REMS compared to the Control. Although L-theanine failed to reverse the caffeine-induced W increase, at 22.5 and 37.5 mg/kg (but not at 75 and 150 mg/kg), it significantly reversed caffeine-induced decreases in SWS. In conclusion, low doses of L-theanine can partially reverse caffeine-induced reductions in SWS; however, effects of L-theanine on caffeine-induced insomnia do not appear to increase dose-dependently.

1. Introduction

Caffeine is one of the most widely used psychoactive substances, best known for its effects as a behavioral stimulant. It is thought to exert its central nervous system effects primarily through adenosine receptor blockade (Deckert and Gleiter, 1989). Caffeine produces a variety of sleep disturbances, including reduction of total sleep time, prolonged latency of sleep onset, and increased wakefulness in humans and rats (Bonnet and Arand, 1992; Karacan et al., 1976; Paterson et al., 2007; Schwierin et al., 1996; Shinomiya et al., 2004). In addition, caffeine administration has been used as a model of insomnia in rats (Bonnet and Arand, 1992; Karacan et al., 1976; Paterson et al., 2007). Previous studies have demonstrated that caffeine intake is a common cause of sleep disruption, and that reducing caffeine intake increases sleep quality (Edelstein et al., 1984; Morgan et al., 1989; Walsh et al., 1986).

Caffeine is usually ingested by drinking coffee or tea. The caffeine contents of coffee and tea in 200 ml serving of instant coffee and same volume of brewed tea are about 80 mg and 40 mg, respectively (Barone and Roberts, 1996). A recent report states that, adults in the UK consume about 240 mg of caffeine a day, mainly from drinking coffee or tea. The caffeine values were: instant coffee 54 mg, ground coffee 105 mg and tea 40 mg per serving (Heatherley et al., 2006). Although coffee and tea provide doses of caffeine sufficient to induce a marked alertness and psychomotor activation, it is commonly considered that drinking tea is less stimulating and more relaxing than drinking coffee. This effect is thought to be due to L-theanine, an amino acid analog present in tea but not in coffee. A cup of tea is estimated to contain 20–50 mg of L-theanine (Rogers et al., 2008). L-theanine has various pharmacological actions such as promoting feelings of calmness, decreasing alertness, and anti-stress effects (Haskell et al., 2008; Kimura et al., 2007). Additionally, Kakuda et al. (2000) showed that by measuring electroencephalography (EEG) in rats intravenous L-theanine at a dose higher than 0.78 mg/kg, could inhibit the stimulatory action of caffeine.

Here, we propose that L-theanine counteracts the effects of caffeine on sleep. Therefore, this study aimed to evaluate the effects of L-theanine on sleep/wake architecture during caffeine-induced sleep-wake disturbance in rats, and to determine whether L-theanine could reverse the sleep-disrupting effects of caffeine.

2. Materials and methods

2.1. Animals

Eight adult male Sprague–Dawley rats (Samtaco, Osan, Korea) were used, weighing 260–320 g at the time of surgery. Animals were individually housed in stainless steel cages (20 cm × 35 cm × 17 cm) throughout

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the study. They were maintained in a controlled environment for the duration of the study: 21–24 °C ambient temperature, 12:12 light–dark cycle (lights on from 7:00 to 19:00), with food (Hyochang Science, Daegu, Korea) and tap water available ad libitum except on the day of the experiment. The experiments were approved by the Kyungpook National University Institutional Animal Care and Use Committee, and were carried out in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (1996). The rats were euthanized by CO_2 overdose after the completion of the experiment.

2.2. Drugs and materials

L-theanine (Sigma-Aldrich Inc., St. Louis, MO, USA) and caffeine (Junsei Chemical Co., Tokyo, Japan) were dissolved in distilled water. The concentration of each drug solution was adjusted so that the volume injected was constant at 1.0 ml/kg body weight. All drugs were prepared daily, and were administered intraperitoneally (i.p.). Previous studies showed that caffeine-induced insomnia was dose-dependent with 7.5 mg/kg i.p. caffeine maintaining wakefulness for at least 2 h (Kwon et al., 2006).

2.3. Procedures

Rats were anesthetized (0.4 mg/kg of medetomidine and 60 mg/kg of ketamine, i.p.), and EEG and EMG recording electrodes were implanted. Once the animals showed no response to tail pinch, they were placed in a stereotaxic apparatus, and a mixture of 2% lidocaine and epinephrine was injected subcutaneously into the scalp to provide local anesthesia and reduce bleeding. An incision was then made in the midline of the scalp, and the skull surface was cleaned. Four small holes were drilled bilaterally into the parietal bones (5.0 mm posterior to and 2.5 mm lateral from bregma) and the interparietal bones (10.0 mm posterior to and 1.25 mm lateral from bregma) using a low-speed burr (ball diameter 0.8 mm) without perforating the dura mater. Gold-plated stainless steel screws (tip diameter 1.1 mm) were screwed tightly into the burr holes. Two screws over the cerebellum (in the interparietal bones) served as reference and ground electrodes. Pins were directly soldered to EMG wires and were connected to screw electrodes using enamel-coated copper wire, and then free-end of pins were arranged to connector in 3×2 matrices and fixed over the skull with dental acrylic. Systemic antibiotics (Cephradine inj., Korea Schnell Pharma Co., Ltd., Gyeonggido, Korea) and analgesics (Butophan inj., Myungmoon Pharmaceutical Co., Ltd., Seoul, Korea) were administered for 2 days, including the day of surgery, to prevent infection and minimize pain during recovery.

After at least one week of recovery, the rats habituated to the experimental procedure at least 2 times before recording. First, the rats were put in the recording box without any treatment from 10:30 to 17:30. Second, the rats were connected to the recording cable without drug treatment. After at least 7 days washout period, the rats were again connected to the recording cable one day before recording. The animals were connected to the recording equipment using a swivel and flexible tether cable system that allowed free movement in the chamber.

On the day of the experiment, the rats were put into the recording chambers at 10:00. After 3.5 h of habituation, the rats received drug injections at 13:20 and 13:30 according to the predetermined treatment plan; saline followed by saline ('Control' group), caffeine (7.5 mg/kg) followed by saline ('CT0' group), or caffeine (7.5 mg/kg) followed by different doses of L-theanine (22.5, 37.5, 75 and 150 mg/kg; groups 'CT1', 'CT2', 'CT3,' and 'CT4', respectively). Electrophysiological activity was then recorded from 13:30 to 17:30. Each rat had at least one week washout period between sessions.

2.4. Recording

Two-channel EEG signals over the parietal cortex were measured in monopolar fashion with respect to the reference electrode. EEG signals were amplified by a factor of 10,000 (Model 3500, A-M Systems, Inc., Carlborg, WA, USA) and bandpass filtered (1 to 500 Hz). A 60-Hz notch filter was also used to remove electric hum. EMG signals were filtered between 1 and 500 Hz and amplified by a factor of 10,000. Analog signals were sampled by an AD converter (DAQ Pad6015, National Instruments Inc., Union City, CA, USA) using the LabView program (National Instruments, Inc., Union City, CA, USA) and digitized at 1 KHz, averaged every five consecutive samples and saved 200 Hz samplings to a disk.

2.5. Evaluation and data analysis

Sleep–wake state was scored manually using 10-s epochs of EEG and EMG activity. The scorer was blind to the substances used. Each

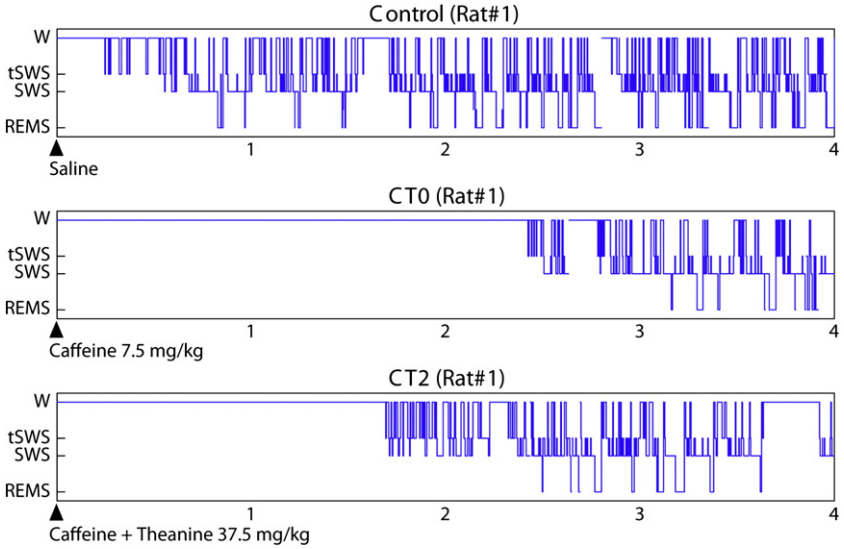

Fig. 1. Representative hypnograms during 4 h after saline (Cont), caffeine (7.5 mg/kg, CT0), caffeine with L-theanine (37.5 mg/kg, CT2) administration. Post-treatment recording was established from 13:30 to 17:30. W, wakefulness; tSWS, transition to SWS; SWS, slow-wave sleep; and REMS, rapid-eye-movement sleep.