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Effects of chlorogenic acid and its metabolites on the sleep–wakefulness cycle in rats

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Abstract

The effect of chlorogenic acid on the sleep–wakefulness cycle in rats was investigated in comparison with those of caffeic acid (the metabolite of chlorogenic acid) and dihydrocaffeic acid (the metabolite of caffeic acid). A significant prolongation of sleep latency was observed with chlorogenic acid and caffeic acid at a dose of 500 and 200 mg/kg, respectively. On the other hand, no remarkable effects were observed with dihydrocaffeic acid even at a dose of 500 mg/kg. Caffeine caused a significant increase in sleep latency and waking time and decrease in non-rapid eye movement sleep time at a dose of 10 mg/kg. In contrast, chlorogenic acid and its metabolites had no significant effects on each sleep state. From these results, it may be concluded that chrologenic acid caused a mild arousal effect compared with that of caffeine, and the effect of chlorogenic acid may have occurred through its metabolite caffeic acid.

Keywords: Sleep latency; Chlorogenic acid; Caffeic acid; Dihydrocaffeic acid; Caffeine; Arousal effect

1. Introduction

Coffee is a central nervous system (CNS) stimulating beverage, and is widely used for the prevention of drowsiness caused by driving a car or deskwork. It is well known that the effects of coffee on the central nervous system are mainly generated by caffeine (George, 2000; Nehlig and Boyet, 2000). On the other hand, caffeine also has many untoward reactions, such as diuresis, increasing blood pressure, various types of arrhythmia and gastrointestinal trouble (Jeong and Dimsdale, 1990; Lynn and Kissinger, 1992; George, 2000). In addition, it seems likely that chronic administration of caffeine for preventing drowsiness is not preferable, because the drug frequently causes loss of sleep at night. Therefore, it seems likely that the use of drugs having a weak stimulating effect is desirable for preventing drowsiness. Chlorogenic acid is known to be contained in coffee beans in large quantities (Clifford, 1975; Trugo and Macrae, 1984). In animal experiments, there have been few reports that chlorogenic acid causes an increase in locomotor activity or CNS stimulation (Czok and Lang, 1961; Valette and Morin, 1969; Ammon and Künkel, 1976). In contrast, Hach and Heim (1971) found that chlorogenic acid caused no influence on motility. Thus, controversy continued as to whether chlorogenic acid has a CNS-stimulating effect or not. We speculate that chlorogenic acid, as well as caffeine, may be associated with the CNS-stimulating effects of coffee. In the present study, therefore, we studied the effect of chlorogenic acid on the sleep–wakefulness cycle in rats in comparison with caffeic acid, the metabolite of chlorogenic acid (Booth et al.,

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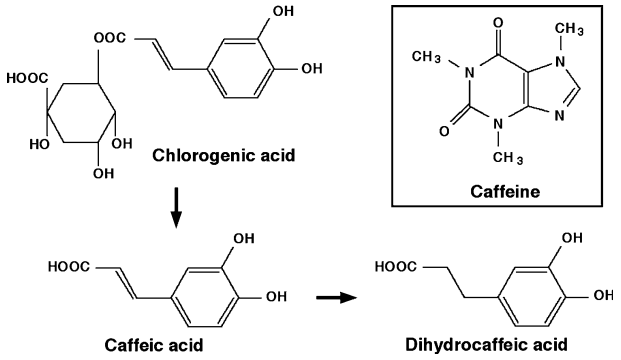

Fig. 1. Chemical structures of chlorogenic acid, caffeic acid, dihydrocaffeic acid and caffeine.

1957; Czok et al., 1974), and dihydrocaffeic acid, the metabolite of caffeic acid (Booth et al., 1957).

2. Materials and methods

2.1. Animals

Thirty-six male Wistar rats weighing 250–310 g (Japan SLC, Shizuoka, Japan) were used. All animals were maintained in an air-conditioned room with controlled temperature (24±2 °C) and humidity (55±15%). They were housed in aluminum cages with sawdust and kept under a light–dark cycle (lights on from 07:00 to 19:00). The animals were allowed free access to food and water except during the experiments. All procedures involving animals were conducted in accordance with the guidelines of the Animal Care and Use Committee, Faculty of Pharmaceutical Sciences, Okayama University.

2.2. Surgery

The animals were anesthetized with pentobarbital sodium (Nembutal®, 35 mg/kg, i.p., Abbott Laboratories, North Chicago, IL, USA), then fixed in a stereotaxic apparatus (SR-5, Narishige, Tokyo, Japan). For electroencephalogram (EEG) recording, a stainless steel screw electrode (200 μm) was chronically implanted into the right frontal cortex (A: 0.5, L: 3.0) according to the atlas of Paxinos and Watson (1986). To record the electromyogram (EMG), stainless steel wire electrodes (200 μm) were implanted into the dorsal neck muscle. The electrodes were connected to a miniature receptacle and the whole assembly was fixed to the skull with dental cement. At least 7 days were allowed for recovery from the surgery.

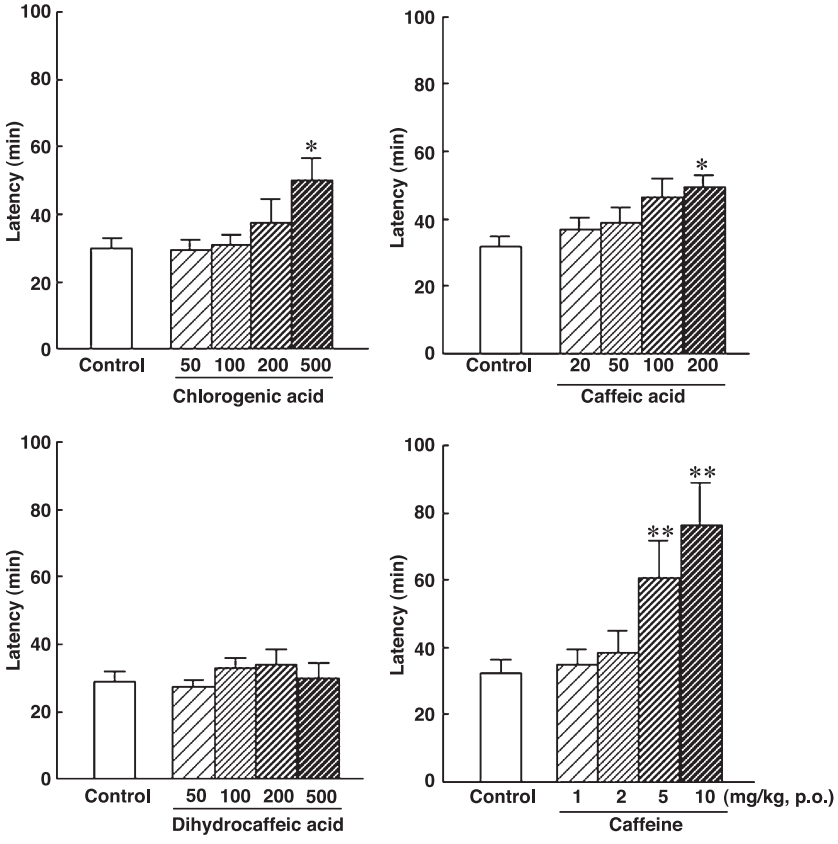

Fig. 2. Effects of chlorogenic acid, caffeic acid, dihydrocaffeic acid and caffeine on sleep latency in rats. Columns and vertical bars represent means±S.E.M. (n=9). Drugs were administered orally. *, **Significantly different from control group at $P<0.05$ and $P<0.01$, respectively.

2.3. EEG and EMG recordings

EEG and EMG were recorded with an electroencephalograph (Model EEG 4314, Nihon Kohden, Tokyo, Japan). The recording was carried out according to the method described previously (Huang et al., 2001; Shinomiya et al., 2004). The signals were amplified and filtered (EEG, 0.5–30 Hz; EMG, 16–128 Hz), then digitized at a sampling rate of 128 Hz and recorded using the data acquisition program SleepSign ver.2.0 (Kissei Comtec, Nagano, Japan). EEG and EMG of the rat were measured in a plastic cage (30×18×24 cm), with its floor covered with sawdust. The observation cage was placed in a sound-proof and electrically shielded box (70×60×60 cm).

2.4. Sleep–wakefulness state analysis

The sleep–wakefulness states were automatically classified by 10-s epochs as wakefulness, non-rapid eye movement (non-REM) or rapid eye movement (REM) sleep by SleepSign ver.2.0 according to the criteria previously described (Shinomiya et al., 2003; Shigemoto et al., 2004). As a final step, defined sleep–wakefulness stages were examined visually, and corrected, if necessary.

2.5. Drugs

The following drugs were used: chlorogenic acid (Sigma, St. Louis, MO, USA), caffeic acid (Sigma), dihydrocaffeic acid (Aldrich, Milwaukee, WI, USA), caffeine (Wako, Osaka, Japan). Chlorogenic acid, dihydrocaffeic acid and caffeine were dissolved in water. Caffeic acid was suspended in 0.5% carboxymethyl cellulose solution. Chemical structures of chlorogenic acid, caffeic acid, dihydrocaffeic acid and caffeine are shown in Fig. 1. The drugs were administered orally, and EEG and EMG were measured for 3 h after drug administration. Nine rats were used in each group, and counterbalanced design for drug dosage was used. Drugs were administered at intervals of 7 days when the same rats were used for repeated experiments. Each rat was subjected to experiment for drug study five times. The doses and molar basis of the drugs used in the study were as follows: chlorogenic acid; 50, 100, 200 and 500 mg/kg (141, 282, 564 and 1411 nmol/kg), caffeic acid; 20, 50, 100 and 200 mg/kg (111, 278, 555 and 1110 nmol/kg), dihydrocaffeic acid; 50, 100, 200 and 500 mg/kg (274, 549, 1098 and 2745 nmol/kg), caffeine; 1, 2, 5 and 10 mg/kg (5, 10, 26 and 52 nmol/kg).

2.6. Data analysis and statistics

Values shown are means±S.E.M. One-way analysis of variance (ANOVA) with the Dunnett's test was used for estimating the drug effects. Sleep latency was defined as the time from drug administration up to the first 12 consecutive 10-s epochs of sleep.

3. Results

3.1. Effects on sleep latency

Chlorogenic acid caused a dose-dependent increase in sleep latency. A significant increase of sleep latency was observed at a dose of 500 mg/kg. Caffeic acid also showed a significant increase in sleep latency at a dose of 200 mg/kg. However, no significant effects on sleep latency were observed with dihydrocaffeic acid. On the other hand, caffeine caused a potent effect on the prolongation of sleep latency, and a significant effect was observed at doses of 5 and 10 mg/kg (Fig. 2).

3.2. Effects on total time of each sleep state

Chlorogenic acid had no significant effects on total times of wakefulness, non-REM sleep or REM sleep even at a dose of 500 mg/kg. Similar findings were also observed with caffeic acid and dihydrocaffeic acid at a dose of 200 and 500 mg/kg, respectively. On the other hand, caffeine at a dose of 10 mg/kg caused a significant increase in total time of wakefulness and a decrease in total time of non-REM sleep (Table 1). Chlorogenic acid, caffeic acid and dihydrocaffeic acid had no effects on the hourly waking, non-REM or REM sleep time. At 0–1 h after administration,

Table 1
Effects of chlorogenic acid, caffeic acid, dihydrocaffeic acid and caffeine on total time of each sleep state in rats

Drugs	Dose (mg/kg)	Wakefulness	Non-REM sleep	REM sleep
Chlorogenic acid	Control	84.7±4.2	85.0±3.1	10.3±1.4
	50	74.6±4.3	91.8±4.5	13.6±2.2
	100	81.0±3.8	85.2±3.9	13.8±2.7
	200	85.9±3.2	82.2±4.5	11.9±1.9
	500	85.9±2.9	83.5±4.1	10.6±1.9
Caffeic acid	Control	92.2±4.7	74.2±4.5	11.8±1.7
	20	91.0±4.1	76.4±3.5	12.7±1.9
	50	88.6±5.9	80.2±5.7	11.2±1.4
	100	102.2±5.2	68.9±4.6	8.9±1.3
	200	96.9±4.8	73.9±5.0	9.2±1.4
Dihydrocaffeic acid	Control	73.5±4.9	96.4±3.3	10.2±2.0
	50	68.8±4.4	97.2±3.8	14.0±1.9
	100	76.1±4.2	92.8±4.5	11.1±1.4
	200	68.0±3.7	101.1±3.4	11.0±1.9
	500	58.4±5.7	109.2±5.0	12.4±1.8
Caffeine	Control	90.7±7.1	77.5±7.3	11.8±2.2
	1	88.7±4.7	78.2±5.1	13.0±1.3
	2	96.3±6.7	70.8±5.2	12.9±2.2
	5	104.1±5.9	67.0±5.7	9.0±1.3
	10	123.2±3.3*	49.7±3.5*	7.2±0.9

Numeral indicates mean minute±S.E.M. Data represent mean±S.E.M. (n=9).
* Significantly different from control group at P<0.01.

Table 2
Effects of chlorogenic acid, caffeic acid, dihydrocaffeic acid and caffeine on hourly wakefulness time in rats

Drugs	Dose (mg/kg)	0–1 (h)	1–2 (h)	2–3 (h)
Chlorogenic acid	Control	35.6±1.7	23.6±1.9	25.6±2.9
	50	33.8±1.5	18.5±2.5	22.3±1.9
	100	36.1±1.2	19.5±2.6	25.5±2.0
	200	35.4±1.3	25.2±2.1	25.3±2.1
	500	39.3±2.2	23.0±1.6	23.6±1.9
Caffeic acid	Control	41.5±1.7	28.5±2.0	23.1±2.6
	20	39.1±2.6	28.4±2.8	26.9±2.7
	50	38.0±2.1	28.3±2.6	23.0±3.7
	100	42.9±2.1	30.5±3.2	30.4±2.3
	200	39.5±2.3	26.8±3.0	30.5±2.6
Dihydrocaffeic acid	Control	33.8±2.3	20.3±1.5	19.4±1.8
	50	30.0±1.7	17.4±2.0	21.4±2.8
	100	36.8±1.4	20.1±2.7	19.1±2.4
	200	33.5±2.5	15.4±1.8	19.0±2.0
	500	28.9±2.8	11.9±2.0	17.6±2.2
Caffeine	Control	38.4±2.5	25.6±3.3	26.7±2.2
	1	40.0±2.1	23.2±2.6	25.5±2.3
	2	41.1±2.7	26.1±3.2	29.0±1.9
	5	47.2±2.3[a]	35.1±3.5	21.8±2.7
	10	55.2±1.9[b]	39.9±3.3[a]	28.1±2.0

Numeral indicates mean minute±S.E.M. Data represent mean±S.E.M. (n=9). [a,b]Significantly different from control group at P<0.05 and P<0.01, respectively.

caffeine at doses of 5 and 10 mg/kg significantly increased the waking time, and decreased the non-REM sleep time compared with control. At 1–2 h after administration, caffeine at a dose of 10 mg/kg caused a significant increase in the wakefulness and decrease in non-REM sleep time. In REM sleep time, a significant decrease was observed 1–2 h after administration of caffeine at doses of 5 and 10 mg/kg (Tables 2–4).

Table 3
Effects of chlorogenic acid, caffeic acid, dihydrocaffeic acid and caffeine on hourly non-REM sleep time in rats

Drugs	Dose (mg/kg)	0–1 (h)	1–2 (h)	2–3 (h)
Chlorogenic acid	Control	21.4±1.4	32.8±1.6	30.7±2.2
	50	24.0±1.5	36.7±2.5	31.1±1.2
	100	21.9±1.1	34.4±2.3	28.8±2.2
	200	22.4±1.4	30.9±1.7	28.9±2.3
	500	19.3±2.4	33.3±1.9	30.9±1.8
Caffeic acid	Control	17.1±1.7	26.4±2.0	29.0±3.2
	20	18.3±2.1	27.3±2.4	27.1±2.5
	50	19.3±2.0	28.0±2.4	31.1±2.8
	100	15.6±1.7	26.2±2.9	24.6±2.2
	200	18.9±2.2	28.8±2.8	26.3±2.4
Dihydrocaffeic acid	Control	24.1±1.9	35.5±0.9	36.8±1.2
	50	26.2±2.0	37.7±1.5	33.3±2.1
	100	22.7±1.5	35.1±2.6	35.1±2.3
	200	23.4±1.8	41.0±1.7	36.7±2.0
	500	28.8±2.4	43.7±1.8	36.7±1.8
Caffeine	Control	20.4±2.1	28.8±3.1	28.3±1.9
	1	17.9±1.8	31.3±2.6	29.0±2.5
	2	16.7±2.0	28.1±2.9	26.0±1.8
	5	12.5±2.2[a]	22.7±3.1	31.8±2.3
	10	4.7±1.8[b]	17.9±2.8[a]	27.1±1.7

Numeral indicates mean minute±S.E.M. Data represent mean±S.E.M. (n=9). [a,b]Significantly different from control group at P<0.05 and P<0.01, respectively.

Table 4
Effects of chlorogenic acid, caffeic acid, dihydrocaffeic acid and caffeine on hourly REM sleep time in rats

Drugs	Dose (mg/kg)	0–1 (h)	1–2 (h)	2–3 (h)
Chlorogenic acid	Control	3.0±0.7	3.6±0.9	3.7±0.8
	50	2.2±0.6	4.9±0.6	6.6±1.4
	100	2.0±0.3	6.1±1.8	5.7±1.2
	200	2.2±0.9	3.9±1.1	5.8±0.7
	500	1.4±0.6	3.7±1.0	5.5±0.8
Caffeic acid	Control	1.4±0.4	5.2±1.0	6.1±0.8
	20	2.6±0.7	4.3±0.8	6.0±0.8
	50	2.7±0.8	3.6±0.5	5.9±1.4
	100	1.4±0.6	3.2±0.8	5.0±0.7
	200	1.6±0.4	4.4±0.8	3.2±0.7
Dihydrocaffeic acid	Control	2.1±0.9	4.2±0.9	3.8±1.0
	50	3.7±1.1	4.9±0.8	5.4±0.9
	100	0.5±0.1	4.8±0.9	5.9±0.9
	200	3.1±1.7	3.6±0.9	4.3±0.9
	500	2.2±0.7	4.5±0.6	5.7±1.2
Caffeine	Control	1.2±0.6	5.6±1.2	5.0±0.9
	1	2.1±0.8	5.5±0.4	5.4±0.6
	2	2.2±1.1	5.8±0.9	5.0±0.6
	5	0.4±0.2	2.2±0.6*	6.4±1.1
	10	0.1±0.1	2.2±0.6*	4.8±0.7

Numeral indicates mean minute±S.E.M. Data represent mean±S.E.M. (n=9).
* Significantly different from control group at P<0.05.

4. Discussion

In the present study, chlorogenic acid caused a significant increase in sleep latency in rats. Sleep latency was studied after the animals were placed in novel cages in which the animals had no access to food and water. Michaud et al. (1982) reported that the total times of non-REM sleep and REM sleep were significantly decreased when rats were moved to a novel individual cage. Roky et al. (1999) also showed a food and water restriction protocol alters the distribution of non-REM sleep and REM sleep in rats. The two factors, novel cage or food and water restriction, may affect the prolongation of sleep latency induced by chlorogenic acid. However, the present findings are not applicable because the sleep latency in control group is also affected by the two factors. In addition, it was found that the prolongation of sleep latency induced by caffeic acid, the metabolite of chlorogenic acid (Booth et al., 1957; Czok et al., 1974), was more potent than that of chlorogenic acid. However, dihydrocaffeic acid, the metabolite of caffeic acid (Booth et al., 1957), had no significant effect on sleep latency. From these results, it seems likely that the prolongation of sleep latency induced by chlorogenic acid may be caused by its metabolite, caffeic acid.

The detailed mechanisms involved in the prolongation of sleep latency induced by chlorogenic acid and caffeic acid are not yet clear. However, Takeda et al. (2002a,b, 2003)

reported that caffeic acid produced antidepressive and anxiolytic-like effects in mice, and the effect of caffeic acid was suppressed by pretreatment with the α_1-adrenoceptor antagonist prazosin. In addition, Cheng and Liu (2000) have revealed that caffeic acid can activate the α_1-adrenoceptor system. It is well known that the arousal effects induced by α_1-adrenoceptor stimulation are reversed by an α_1-antagonist (Monti, 1982; Pellejero et al., 1984; Guo et al., 1991). From these findings, the α_1-adrenoceptor system may contribute to the arousal effects of caffeic acid. On the other hand, caffeine caused a potent prolongation of sleep latency even at a dose of 5 mg/kg. It is well known that the CNS stimulant properties of caffeine are due to reduction of adenosine transmission in the brain (Fisone et al., 2004). Therefore, it may be that the mechanism of the CNS-stimulant effects of chlorogenic acid is quite different from that of caffeine.

In addition, caffeine at a dose of 10 mg/kg caused a potent and significant increase in total waking time and decrease in total non-REM sleep time. In contrast, no significant effects were observed with chlorogenic acid and caffeic acid on total time of each sleep state. These results suggest that the arousal effects of chlorogenic acid and caffeic acid had no obvious effect on the sleep–wakefulness cycle, even though the two compounds produced a significant increase in sleep latency. Thithapandha et al. (1972) showed that the biologic half-life of intravenous administration of caffeine in plasma was 4.2 h in rats. On the other hand, Takenaka et al. (2000) reported that more than 90% of chlorogenic and caffeic acid disappeared from plasma within 30 min after intravenous injection into rats. These findings seem to explain why chlorogenic and caffeic acid caused no change significantly the total times of wakefulness, non-REM sleep or REM sleep.

In conclusion, chlorogenic acid and caffeic acid may be more useful than caffeine for preventing the drowsiness especially in hypertension, arrhythmia and gastrointestinal ulcer patients in whom caffeine treatment causes problems.

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