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Research report

Stimulation of A_1 adenosine receptors mimics the electroencephalographic effects of sleep deprivation

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Abstract

N^6-Cyclopentyladenosine (CPA), an A_1 adenosine receptor agonist, increased EEG slow-wave activity in nonREM sleep when administered either systemically (0.1–3 mg/kg) or intracerebroventricularly (3.5–10 μg) in the rat. The power spectrum of EEG changes (as calculated by Fourier analysis) matched that produced by total sleep deprivation in the rat. The effects of CPA on the nonREM-sleep EEG were dose-dependent. These findings suggest that adenosine is an endogenous mediator of sleep-deprivation induced increases in EEG slow-wave activity, and therefore that increased adenosine release is a concomitant of accumulation of sleep need and may be involved in homeostatic feedback control of sleep expression.

Keywords: Potassium conductance; Fourier analysis; Delta power; N^6-Cyclopentyladenosine; EEG; Sleep homeostasis; Sleep function

1. Introduction

The intensity of slow-wave activity (SWA) in the cortical electroencephalograph (EEG) during non-rapid eye movement (nonREM) sleep is the most consistent physiological index of sleep homeostasis [7,12,30]. EEG SWA in nonREM sleep increases as a function of duration of prior waking and decreases monotonically during sleep, suggesting that accumulated sleep propensity potentiates the action of the neuronal generators of slow EEG waves. This connection between accumulated sleep propensity and nonREM-sleep EEG SWA provides an avenue for determining the physiological basis of sleep homeostasis, hence the function of sleep. By tracing the pathway whereby nonREM-sleep EEG SWA is potentiated, one may gain insight into the neurochemical condition that underlies accumulated sleep propensity.

EEG SWA is a product of synchronized bursting activity in neurons of the cerebral cortex and thalamus [9,36]. Rhythmic bursting in the 0.5–4.0 Hz frequency range is present when neuronal resting membrane potential is hyperpolarized to more than ca. −70 mV [11]. Further hyperpolarization increases the rhythmicity of the bursting and facilitates synchronization in neuronal populations [29]. The optimal membrane potential range for neuronal synchronization is more negative than the ca. −70 mV reversal potential for Cl^-. The reversal potential for K^+, by contrast, is ca. −90 mV. Increased modulatory GK^+ is therefore the most likely mechanism underlying the membrane hyperpolarization responsible for potentiating EEG SWA.

Adenosine increases GK^+ in the cerebral cortex by stimulation of A_1 adenosine receptors [21], which are fairly homogeneously distributed throughout the cerebral cortex and thalamus [13,15]. The effect is G-protein mediated and primarily involves an increase in a 'leak' K^+ conductance [35]. Another major effect of adenosine receptor activation is inhibition of neurotransmitter release (reviewed in [17]). Adenosine is ideally suited to potentiate synchronization of neuronal activity in the cerebral cortex and thalamus, hence EEG SWA in nonREM sleep, in that it increases GK^+ and decreases depolarizing inputs. We have therefore hypothesized that adenosine released as a function of accumulated sleep debt produces the sleep-debt related changes in EEG SWA in nonREM sleep [4].

To test the hypothesis that stimulation of A_1 adenosine receptors potentiates EEG SWA, we have administered N^6-cyclopentyladenosine (CPA), a highly selective A_1 agonist [34], either systemically or intracerebroventricularly (i.c.v.) to adult male Wistar rats and quantified the resul-

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tant changes in EEG SWA using Fourier analysis. These results have appeared previously in abstract form [5,19].

2. Materials and methods

Adult male Wistar rats, weighing 250–310 g at time of surgery were instrumented with epidural EEG and nuchal EMG electrodes under anesthesia (ketamine 80 mg/kg, xylazine 8 mg/kg, and acepromazine maleate 1.6 mg/kg administered intraperitoneally with intramuscular supplements as necessary). Details of EEG implantation procedures are described elsewhere [6]. Guide cannulae for i.c.v. injections (22 gauge stainless steel tubing, manufactured by Plastics One) were directed at the lateral ventricle (posterior 0.8 mm, lateral 1.5 mm, ventral −3.6 mm from bregma). The guide cannulae were blocked by obturators until immediately before i.c.v. injections. Animals were given an intramuscular 0.25 ml gentamicin antibiotic injection after surgery and monitored until recovery from anesthesia.

Animals were allowed at least 7 days recovery from surgery and were then acclimated to the recording environment for at least 72 h. Animals were housed individually in transparent Plexiglas cages within electrically grounded Faraday cages. One EEG electrode was used to ground the animal. This set-up allowed EEG recording with negligible movement artifact. The recording room was sound-attenuated, dimly lit (lights on 12 h per day, between 08:00 and 20:00), and maintained at 22°C. Animals were given food and water ad libitum.

Animals were cabled via a commutator to a Grass polygraph. Frontal-parietal EEG from one hemisphere was filtered at 0.3 Hz and 35 Hz, digitized at 100 Hz and stored in 10-s epochs on a PC. EEG was Fourier analyzed in 10-s epochs and spectral power was averaged in 0.5 Hz bins from 0–5 Hz and 1 Hz bins from 5–20 Hz. For purposes of scoring sleep/wake states, spectral power was averaged in three frequency bands: delta (0.5–4 Hz), theta (6–9 Hz) and sigma (10–14 Hz). EMG was full-wave rectified, integrated with a 0.5 s time-constant, and stored as one value (0–100) per epoch. Data were collected 24 h per day except for 1 h every 4–8 days for backing up data from the computer's hard disk. Sleep/wake states and transitions from nonREM sleep to REM sleep (NRTs) were scored algorithmically according to the above electrographic indices as described previously [6]. In each animal, the accuracy of algorithmic scoring of sleep/wake states during both control and CPA-treated recordings was confirmed by visual examination of EEG power values, using the criteria described in [6]. The animal-specific thresholds used produced accurate state scoring during both control and CPA-treated recordings in all cases.

EEG power values were expressed as percentage of time-matched baseline recordings. From each animal, 48–120 h of baseline recordings, isolated from any treatments by at least 72 h and interspersed throughout the period of recording from that animal, were collected and averaged in 1-h bins by time of day. Average power values from the set of baseline recordings from each animal were examined to confirm that no systematic changes in power had occurred during the recording period. All baseline recordings were conducted at 22°C. Normalizing EEG power values to animal-specific baseline values reduces inter-subject variability in absolute power that results from electrode manufacture and placement and cabling connections.

CPA (Sigma) was administered i.p. in a vehicle of 0.2 ml DMSO or i.c.v. in a vehicle of 2–5 μl artificial cerebrospinal fluid. i.c.v. doses of CPA were administered at a rate of 1 μl/min. The above volume of DMSO (administered i.p.) has been shown previously to have no effect on sleep expression [2]. All treatments were administered 4 h after the beginning of the rest period ($\pm$30 min), hence after the peak in EEG SWA that begins the rest period [33]. Successive treatments in a given animal were administered in random order and spaced at least 96 h apart.

Some systemic CPA treatments were conducted at a higher ambient temperature. For those treatments, animals were transferred 2 h prior to drug or vehicle administration, to a chamber (measuring 41 cm by 12 cm) with water-perfused walls. They had previously been adapted to this chamber. A perfusion temperature of 32°C was chosen because trials had shown that at this temperature, brain temperature is maintained within 0.5°C of baseline (mean brain temperature during the 2 h before transfer to 32°C) following all treatment doses.

All statistical tests were calculated using SAS, v. 6.03. Two-way ANOVAs for power spectra and time-course were calculated with repeated measures for the frequency and hour variables, respectively. All post-hoc tests are Fisher's LSD tests, using significance thresholds of $P < 0.05$.

3. Results

The behavioral effects of CPA were manifested within minutes of administration and consisted of lethargy and muscle flaccidity. CPA administered systemically to rats maintained at normal room temperature (22 ± 2°C) produced a dose-dependent decrease in brain temperature (taken as a measure of deep body temperature). Brain temperature decreased by 1–2°C for ca. 2 h following 1 mg/kg CPA and by 5–6°C for ca. 6 h following 3 mg/kg CPA. Brain temperature did not decrease following i.c.v. administration of CPA, suggesting that the thermoregulatory effects of CPA are peripherally mediated, probably a result of decreased heat generation in muscle tissue. To compensate for decreased heat generation, systemic administration of CPA was also performed at an upper thermoneutral ambient temperature of 32°C. At this ambient

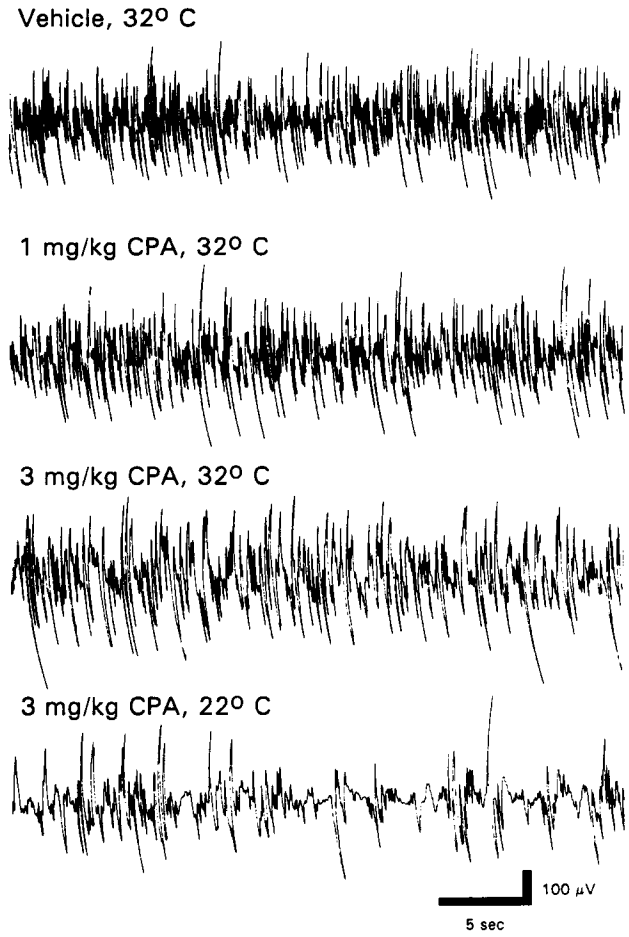

Fig. 1. Examples of nonREM-sleep EEG recordings following CPA administration. Each trace represents 34 s of EEG during deep nonREM sleep circa 3 h following treatment (±15 min). The top three traces are recorded at 32°C, the bottom trace at 22°C. Calibration bars are shown at the bottom.

temperature, brain temperature was maintained within 0.5°C for the entire post-treatment period after vehicle treatment and all doses of CPA.

CPA increased EEG delta power (0.5–4 Hz, a measure of SWA) in nonREM sleep when administered i.p. at both 22°C and 32°C and when administered i.c.v. Examples of the nonREM-sleep EEG ca. 3 h after administration of two doses of CPA and vehicle are shown in Fig. 1. Increased prevalence of slow EEG waveforms is present after 1 mg/kg CPA at 32°C and is more pronounced after 3 mg/kg CPA at 32°C. In both cases, the EEG resembles an intensification of the normal nonREM-sleep EEG. At 22°C, 3 mg/kg CPA produced an EEG pattern that differs from the normal nonREM-sleep EEG. This pattern, which is associated with hypothermia [16], presumably results from the pronounced decrease in brain temperature that follows this treatment.

The time courses of changes in nonREM-sleep EEG delta power following i.p. administration of 1 mg/kg CPA and 3 mg/kg are shown in Fig. 2. Because administration of 3 mg/kg CPA at 22°C produces large decreases in brain temperature and abnormal EEG patterns, the 3 mg/kg treatments shown are those administered at 32°C (compared to vehicle treatments at 32°C). Peak increases in EEG delta power occurred during the period 2–6 h following administration. Although nonREM-sleep EEG delta power was not consistently increased relative to vehicle-treated animals until 2 h after CPA administration, single episodes of high delta-power nonREM sleep were always seen following CPA administration at 32°C. Following 3 mg/kg doses of CPA, EEG delta power during these episodes was significantly increased (by 76.4%) versus nonREM-sleep in vehicle-treated animals during the first hour following treatment. Therefore, administration of CPA

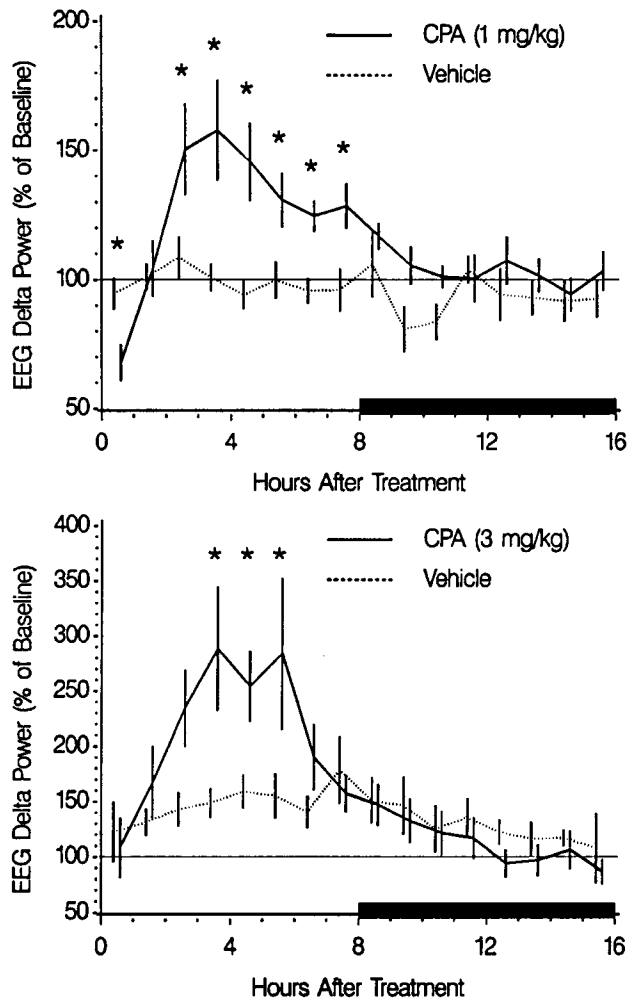

Fig. 2. NonREM-sleep EEG delta power is increased following ip administration of 1 mg/kg CPA and 3 mg/kg CPA versus vehicle controls. Values are hourly means and S.E.M. Solid bar at bottom of display delimits the lights-off period. In two-way ANOVAs (dose × hour), there is a significant main effect of dose ($P < 0.02$, 1 mg/kg), significant main effects of hour ($P < 0.001$, 1 mg/kg and 3 mg/kg), and significant (dose × hour) interactions ($P < 0.005$, 1 mg/kg and 3 mg/kg). Stars denote hours that are significantly different from vehicle ($P < 0.05$). In panel A treatments, ambient temperature is 22°C. In panel B treatments, ambient temperature is 32°C.

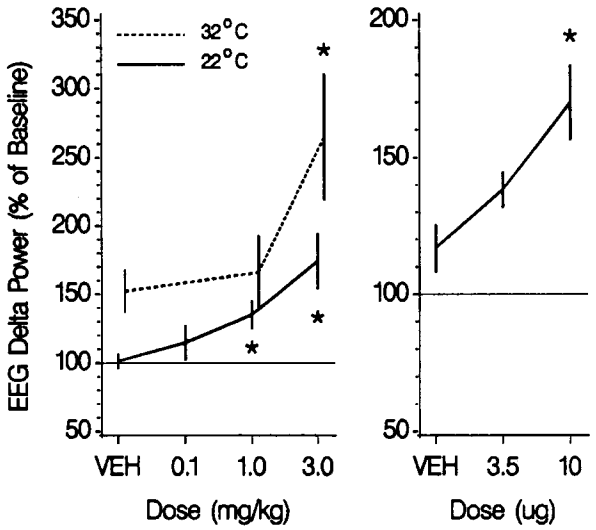

Fig. 3. Dose–response curves for effects on nonREM-sleep EEG delta power of systemically and centrally administered CPA. Values are means of EEG delta power in nonREM sleep for the period from 2–6 h following CPA or vehicle administration. Panel A shows the results of systemically administered CPA at two ambient temperatures. In a two-way ANOVA (dose × ambient temperature), there are significant main effects of both dose and temperature ($P < 0.005$). There is a significant effect of dose in each temperature group (one-way ANOVA, $P < 0.002$ for 22°C trials and $P < 0.05$ for 32°C trials). Stars denote doses that are significantly different from vehicle in each temperature group ($P < 0.05$). Panel B shows the results of CPA administered i.c.v. There is a significant effect of dose (one-way ANOVA, $P < 0.05$). Stars denote doses that are significantly different from vehicle ($P < 0.05$).

immediately promoted generation of EEG slow waves, even though episodes of consolidated high-delta nonREM sleep did not occur until somewhat later.

The increase in nonREM-sleep EEG delta power produced by systemically administered CPA was both dose-dependent and temperature-dependent (Fig. 3A). In both temperature groups, CPA increased nonREM-sleep EEG delta power versus control. The increase in nonREM-sleep EEG delta power was higher in the high ambient temperature group at all doses, including control. CPA administered i.c.v. also produced a dose-dependent increase in nonREM-sleep EEG delta power (Fig. 3B).

As shown in Fig. 3A, there was a small increase in EEG delta power over the baseline level in control trials at 32°C ambient temperature. Two effects of introduction to the 32°C ambient temperature chamber may account for this increase. There was an increase in waking time versus time-matched baseline recordings during the 2-h acclima-

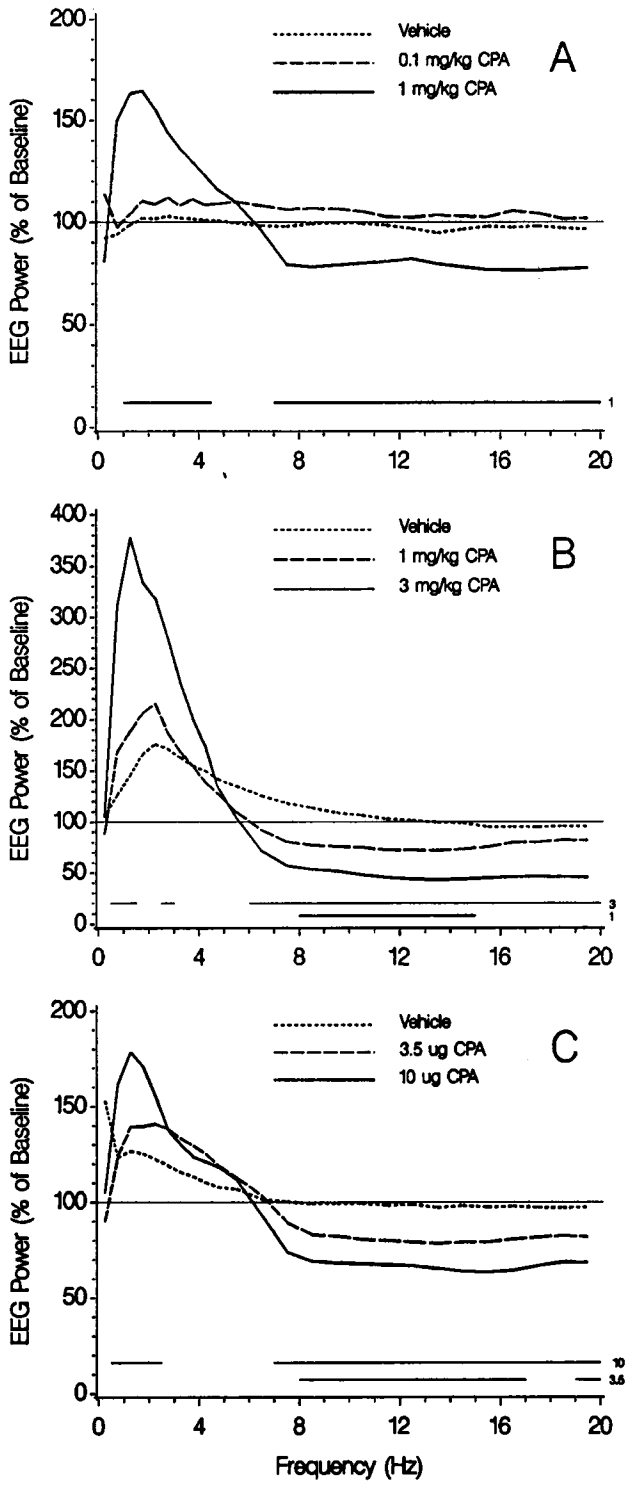

Fig. 4. Power spectrum of changes in the nonREM-sleep EEG produced by CPA administration. EEG power is calculated in 0.5 Hz bins from 0 to 5 Hz and in 1.0 Hz bins from 5 Hz to 20 Hz for the period 2–6 h after treatment. All values are expressed as percentage of EEG power in each frequency bin in time-matched baseline recordings. Panel A shows the results of systemically administered CPA at 22°C ambient temperature. In a two-way ANOVA (dose × frequency), there is a significant main effect of frequency ($P < 0.001$) and a significant (dose × frequency) interaction ($P < 0.001$). Panel B shows the results of systemically administered CPA at 32°C ambient temperature. In a two-way ANOVA (dose × frequency), there is a significant main effect of frequency ($P < 0.001$) and a significant (dose × frequency) interaction ($P < 0.001$). Panel C shows the results of CPA administered i.c.v. In a two-way ANOVA (dose × frequency), there is a significant main effect of frequency ($P < 0.001$) and a significant (dose × frequency) interaction ($P < 0.02$). Lines at bottom of each panel denote frequencies that are significantly different from vehicle for each dose ($P < 0.05$).

Table 1
Effects of CPA on sleep/wake state expression

Treatment	Waking	NonREM sleep	REM sleep
Vehicle, i.p., 22°C ($n = 8$)	34.8 (3.2)	53.3 (3.2)	11.9 (0.6)
0.1 mg/kg, i.p., 22°C ($n = 3$)	39.2 (16.0)	49.4 (11.4)	11.4 (5.6)
1 mg/kg, i.p., 22°C ($n = 7$)	46.7 (2.9) *	50.9 (3.3)	2.4 (1.1) * *
3 mg/kg, i.p., 22°C ($n = 5$)	50.7 (3.0) *	49.3 (3.0)	0.0 (0.0) * *
Vehicle, i.p., 32°C ($n = 6$)	33.7 (7.7)	55.8 (5.7)	10.5 (4.0)
1 mg/kg, i.p., 32°C ($n = 6$)	26.4 (2.8)	70.4 (2.8) *	3.2 (1.0)
3 mg/kg, i.p., 32°C ($n = 6$)	10.5 (1.2) *	89.2 (1.3) * *	0.2 (0.1) *
Vehicle, i.c.v. ($n = 5$)	22.1 (1.7)	64.0 (1.7)	14.0 (0.5)
3.5 μg, i.c.v. ($n = 5$)	26.9 (2.5)	64.5 (1.9)	8.6 (1.8) *
10 μg, i.c.v. ($n = 6$)	42.5 (5.8)	54.7 (5.2)	2.8 (1.4) *

Values are mean (S.E.M.) percentage of recording time during the first 6 h after treatment. Statistical comparisons for each state employed two-tailed Student's t-tests vs. same-condition vehicle treatment. * $P < 0.05$; * * $P < 0.001$.

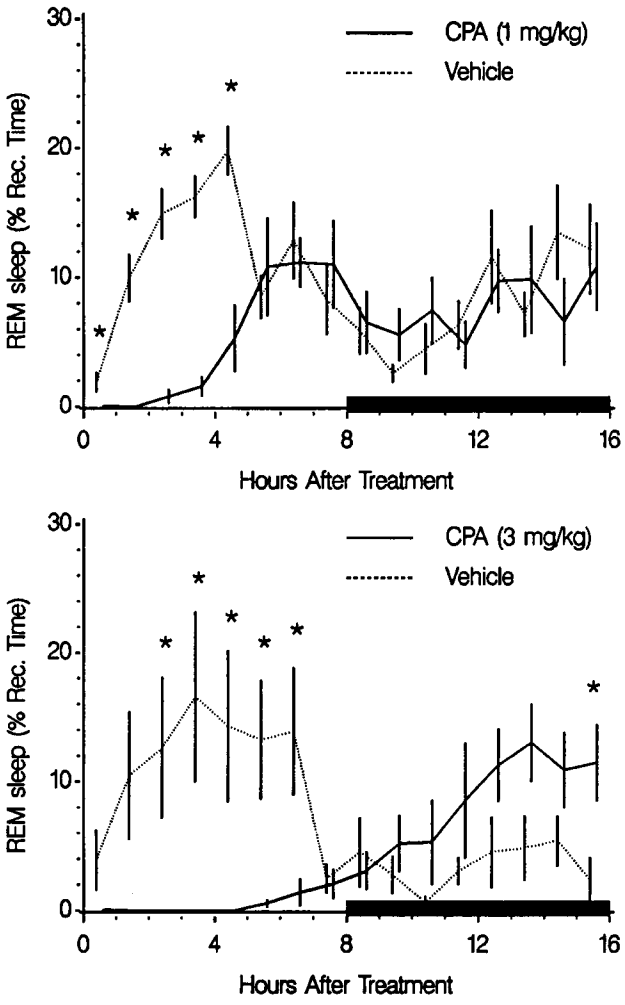

Fig. 5. REM-sleep expression following i.p. administration of 1 mg/kg CPA and 3 mg/kg CPA versus vehicle controls. Values are hourly means ± S.E.M. Solid bar at bottom of display delimits the lights-off period. In two-way ANOVAs (dose × hour), there is a significant main effect of dose ($P < 0.01$, 1 mg/kg), a significant main effect of hour ($P < 0.001$, 1 mg/kg), and significant (dose × hour) interactions ($P < 0.001$, 1 mg/kg and 3 mg/kg). Stars denote hours that are significantly different from vehicle ($P < 0.05$). In panel A treatments, ambient temperature is 22°C. In panel B treatments, ambient temperature is 32°C.

tion period before drug or vehicle treatment (83.4 vs. 41.0 min), and brain temperature transiently increased by 0.7–1.1°C in the first hour of exposure to the higher ambient temperature (increased slow-wave sleep as a result of elevated brain temperature has previously been reported [22]). By the time of administration of CPA or vehicle, brain temperature was within 0.5°C of baseline and remained within that window for the next 7 h.

The power spectrum of changes in the nonREM-sleep EEG produced by CPA administration is shown in Fig. 4. Panels A, B, and C show data from systemic administration at 22°C and 32°C, and from i.c.v. administration, respectively. The amplitude of the response differed among treatment conditions, but the spectral profile was conserved. In all cases, power was increased at low frequencies (< 5 Hz), the peak increase being at 1–2 Hz, and was slightly decreased at higher frequencies (> 6 Hz). The magnitude of both the increase in low frequencies and the decrease in high frequencies was dose dependent.

The effects on sleep/wake state expression of all CPA treatments are shown in Table 1. In addition, the time-course of changes in REM-sleep expression produced by 1 mg/kg and 3 mg/kg i.p. are shown in Fig. 5 (as in Fig. 2, the 3 mg/kg treatments shown were administered at 32°C). The most marked effect of CPA on sleep/wake state expression was a dose-dependent decrease in REM-sleep expression. Following the 3 mg/kg dose i.p., there was a rebound increase in REM sleep during the subsequent active period (significant only in the last hour of the analysis, see Fig. 5B). CPA increased waking expression when administered i.p. at 22°C but decreased waking and increased nonREM-sleep expression when administered i.p. at 32°C.

4. Discussion

CPA, a highly selective A_1 adenosine receptor agonist, produced dose-dependent increases in EEG SWA in non-REM sleep when administered systemically or i.c.v. When administered systemically, CPA also decreased brain temperature (as a measure of core temperature), but CPA-induced increases in nonREM-sleep EEG SWA appear to be independent of the decrease in brain temperature, in that they occurred when brain temperature was maintained at baseline levels by exposure to a higher ambient temperature and when CPA was administered i.c.v., in which case brain temperature was not changed.

Not only did CPA increase nonREM-sleep EEG SWA, but the power spectrum of change in the nonREM-sleep EEG closely matches that produced by TSD in rats (see Fig. 3, cf. Fig. 2 in [31] and Fig. 3 in [14]). Other pharmacological treatments (e.g. the 5-HT_2 antagonist ritanserin in normal rats and the benzodiazepine triazolam in SCN-lesioned rats following sleep deprivation) have previ-

ously been reported to increase EEG delta power, but the power spectra of changes in the EEG produced by these treatments do not match that produced by TSD [8,32], suggesting that the sleep-deprivation related potentiation of EEG SWA is not mediated by 5-HT_2 or $GABA_A$ receptors.

The effects of CPA administration differ from those of TSD in two respects. First, the magnitude of decrease in nonREM-sleep EEG power at high frequencies was somewhat greater than in recovery from TSD, particularly following higher doses of CPA (3 mg/kg i.p. and 10 μg i.c.v., see Fig. 3, cf. Fig. 2 in [31] and Fig. 3 in [14]). This difference between CPA-induced changes and sleep deprivation-induced changes in EEG power spectra is, however, trivial by comparison to the very different power spectrum that results from, e.g., triazolam administration [32]. Second, CPA markedly suppressed REM-sleep. In humans, REM-sleep expression is reduced immediately following TSD (for review, see [3]), but not as markedly as in these experiments. In animals, REM-sleep expression is actually increased following TSD (for review, see [3]). We have elsewhere hypothesized that increased REM-sleep expression following TSD in experimental animals results from the incidental occurrence of nonREM sleep and nonREM sleep-like neuronal activity during the TSD period and is therefore unrelated to accumulation of sleep need per se [3]. However, there is no evidence in humans or animals of REM-sleep suppression on the order of that produced by CPA. The actions of systemically administered CPA may differ from that of endogenous adenosine in that the rate of endogenous adenosine release may vary over the course of the sleep cycle, being high in nonREM sleep and low in REM sleep (see [4]). Experimental stimulation of adenosine receptors by pharmacological treatment may interfere with REM-sleep expression because adenosinergic tonus remains high in the transition to REM sleep, thereby disrupting the active inhibitory mechanisms that facilitate maintenance of REM sleep (reviewed in [28]).

The effects of CPA on nonREM-sleep EEG SWA are in agreement with previous studies using A_1 adenosine agonists and other adenosine-related agents in rats, which found selective increases in S2 nonREM sleep, the stage of nonREM sleep in which EEG slow waves predominate [24–26]. However, the effect of CPA on REM-sleep expression differs from the effects of A_1 adenosine agonists used in previous studies, which did not suppress REM sleep. This difference in effect may result from the fact that the dose of CPA used in the present study was somewhat larger in relation to the affinity of CPA for the A_1 adenosine receptor [34] than were the doses of A_1 agonists used in previous studies. Recently, caffeine administered to humans, a treatment that blocks A_1 adenosine receptors, has been shown to decrease nonREM-sleep EEG SWA, producing a power spectrum of change that is roughly the inverse of that produced by CPA administration [20]. This finding lends support to the supposition that the effects of CPA in the present study are mediated by stimulation of A_1 adenosine receptors.

The fact that CPA produces changes in the nonREM-sleep EEG that closely match those produced by TSD suggests that endogenous adenosine mediates the sleep-homeostatic modulation of the nonREM-sleep EEG. This conclusion implies that adenosine release is increased following sleep deprivation. We believe that the effects of adenosine on nonREM-sleep EEG SWA occur predominantly via direct effects of adenosine on neurons in the cerebral cortex and thalamus. Subsequent to our original presentation of this hypothesis [5,19], Rainnie et al. [27] have proposed as an alternative that the effects of adenosine on sleep propensity and nonREM-sleep EEG SWA are mediated by inhibition of brainstem cholinergic neurons.

It is important to note that an increase in stimulation of adenosine receptors must entail an increase in the rate of adenosine release, rather than accumulation of adenosine during waking, because adenosine is metabolized rapidly [1,10,18,23]. In this respect, adenosine differs from the classical conception of a sleep factor. We have elsewhere presented our model of the role of adenosine in sleep homeostasis in more depth than is possible here [4]. We therefore propose that increased adenosine release operates as a feedback signal, stimulated by depletion of cerebral glycogen reserves. In this model, glycogen depletion is the neurochemical condition that arises during waking and necessitates sleep to permit restoration, and is therefore the physiological basis of sleep need.

Acknowledgements

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