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Role of Adenosine in Sleep and Temperature Regulation in the Preoptic Area of Rats

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TICHO, S. AND M. RADULOVACKI. *Role of adenosine in sleep and temperature regulation in the preoptic area of rats.* PHARMACOL BIOCHEM BEHAV **40**(1) 33–40, 1991.—We have examined the effects on sleep and brain temperature of bilateral microinjections of adenosine and adenosine analogs to the preoptic area (PO) of rats. Administration of adenosine (12.5 nmoles), a nonselective adenosine A1/A2 receptor agonist NECA (N-ethyl-carboxamido-adenosine, 1.0 nmole), and the selective adenosine A1 receptor agonist CPA (cyclopentyladenosine, 0.25, 0.5 nmoles) increased total sleep primarily through an enhancement in deep slow-wave sleep (SWS_2), while adenosine also increased REM sleep. Administration of 12.5 nmoles adenosine and 0.25 nmoles CPA did not affect brain temperature, while 1.0 nmole NECA and 0.5 nmoles CPA caused a transient and prolonged hypothermia, respectively. Administration of the selective adenosine A2 receptor agonist CV-1808 (2-phenylaminoadenosine, 5, 10 nmoles) had no effect on sleep or brain temperature. The present results demonstrate a site for the central hypnotic action of adenosine, and a functional role for adenosine A1 receptors in the hypothalamus.

Preoptic area Adenosine N-Ethyl-carboxamido-adenosine (NECA) Cyclopentyladenosine (CPA) 2-Phenylaminoadenosine (CV-1808) Brain temperature Microinjection Sleep Adenosine A1 receptors Rats

ABUNDANT evidence has accumulated supporting a role for adenosine as a neuromodulator in the CNS (33,39). In addition to its biochemical and electrophysiological actions, adenosine has potent sedative, analgesic, hypnogenic, and anticonvulsant effects (3). For instance, when administered to several animal species, adenosine produced sedation and sleep (9, 11, 16, 35), including increases in both slow-wave (SWS) and rapid-eye movement (REM) sleep (35). Little is known, however, about the site(s) at which adenosine induces these effects. In 1918, Von Economo, while studying patients with encephalitis lethargica, postulated the existence of a sleep center located in the basal forebrain (10). Later, it was shown that lesions slightly rostral to the preoptic area (PO) increased wakefulness (19, 31, 43) and reduced both SWS and REM sleep (42), while chemical and electrical stimulation of the PO induced sleep (37,40). In fact, a complex interrelationship of thermoregulatory and sleep controls within the PO has been reported (8,41). For example, sleep disturbances produced by basal forebrain damage in rats can be attenuated by manipulation of the ambient temperature (43), while adrenergic stimulation has been reported to produce hypothermia as well as arousal (8).

Although little is known of the physiological role of adenosine in the hypothalamus, the abundance of 5-nucleotidase, an adenosine producing enzyme, adenosine deaminase, an adenosine degrading enzyme (27–29), and adenosine uptake sites (14, 20, 27), suggests an important functional role for adenosine in that brain area. Based on pharmacological studies, extracellular receptors for adenosine have been classified as A1 and A2 (44). Autoradiographic studies have shown a heterogeneous distribution of adenosine A1 receptors throughout the CNS with low densities in the hypothalamus and brainstem and higher densities in the striatum, hippocampus, cortex, and cerebellum (15,23). Adenosine A2 receptors, on the other hand, are absent from the hypothalamus, having high densities specifically localized only to the olfactory tubercle, nucleus accumbens, globus pallidus, and striatum (17).

The present studies were undertaken to examine whether adenosine in the preoptic area may be involved in the regulation of sleep as well as temperature in rats. In addition, we investigated the roles of A1 and A2 adenosine receptors in the mechanism of adenosine action in the PO. For that purpose, in addition to adenosine, three metabolically stable adenosine analogs were administered to the PO: a nonselective adenosine A1/A2 receptor agonist NECA, a selective adenosine A1 receptor agonist CPA, and a selective adenosine A2 receptor agonist CV-1808 (5).

METHOD

Animals

Adult male Sprague-Dawley rats weighing 300 g were obtained from Sasco Animal Laboratories (Omaha, NE). Animals were maintained on a 12-h light (0800–2000 h)/12-h dark (2000–0800) cycle, at an ambient temperature of 22 ± 1°C. Rats were housed in individual cages with an ad lib access to food and water throughout the experiment.

Surgery

The surgical method used in this study has been published previously (36) and will be briefly described here. Animals were

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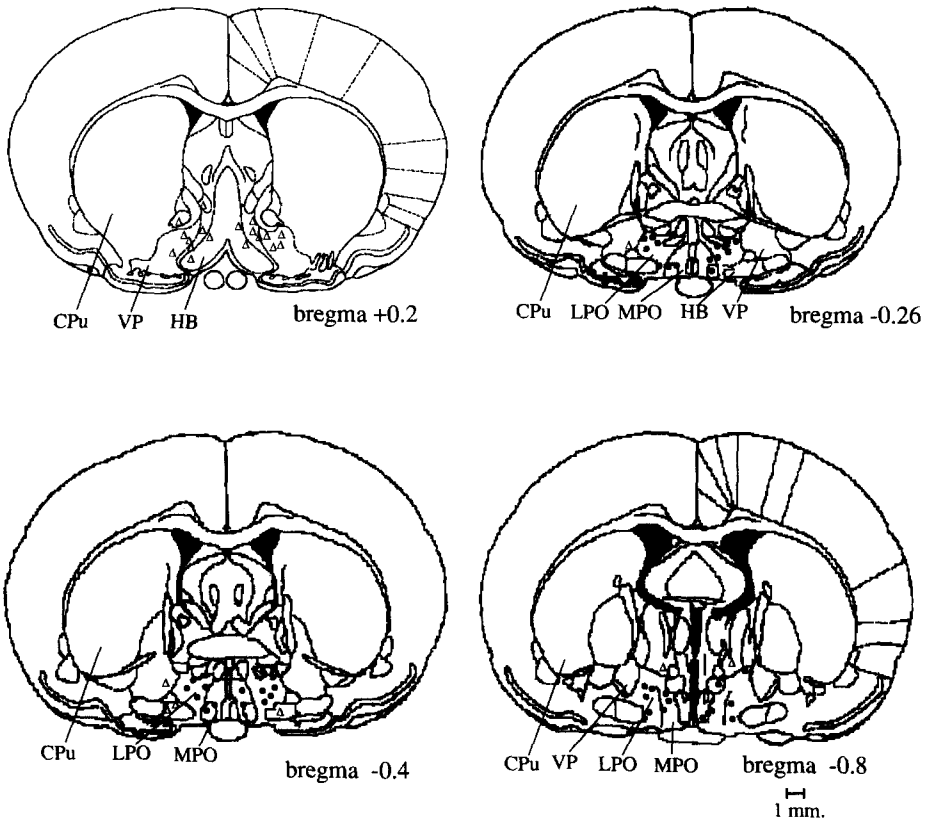

FIG. 1. Anatomical mapping of bilateral injection sites in coronal sections AP 0.2 to AP −0.8 depicting injections within the preoptic area (IN PO: •) and around the preoptic area (OUT PO: △). CPu: caudate-putamen; HB: diagonal band; LPO: lateral preoptic area; MPO: medial preoptic area; VP: ventral pallidum. Diagrams adapted from (32).

anesthetized for the implantation of electrodes using a mixture of ketamine (Vetalar 100 mg/ml)/acetylpromazine (10 mg/ml) (4:1 v/v) at a volume of 1 ml/kg. The surface of the skull was exposed and cleaned with a 20% solution of hydrogen peroxide followed by a solution of 95% isopropyl alcohol. Next, a dental preparation of sodium fluoride (Flura-GEL, Saslow Dental, Mt. Prospect, IL) was applied to cover the screw heads and surrounding skull to further promote the adhesion of the implant. EMG electrodes consisted of 2 ball-shaped wires which were inserted into the bilateral neck musculature. All leads were then soldered to a miniature connector (ER-7S-6, Microtech Inc., Boothwyn, PA). In addition, bilateral guide cannulae (22-ga stainless steel tubes, 25 mm in length) were stereotaxically placed 1 mm above the PO utilizing the following stereotaxic coordinates: AP = −0.4 mm, L = −1.0 mm, DV = 7.3 mm below the dura mater (32). A blocking stylet was placed in each guide cannula at the time of surgery. A 36-ga type T thermocouple (Omega, Stamford, CT) was secured to the left of the left cannula and inserted ventrally to DV = 4.0 to record brain temperature (T_{br}). This method has previously been shown to be a good indicator of core temperature in an unrestrained rat (22). The entire implant assembly (EEG and EMG recording electrodes, electrical connectors, guide cannulae, and thermocouple wire) was anchored to the skull with dental acrylic. After surgery, all animals were individually housed and allowed a one-week recovery period before use in the study.

Experimental Paradigm

All drug solutions were prepared in pyrogen-free saline, except CV-1808. CV-1808 was prepared by dissolving in a 1:1 solution of ethanol and Emulphor polyoxyethylated vegetable oil (generous gift of J. V. Martin) and then diluting with nine parts of artificial cerebrospinal fluid. The injector system consisted of a 28-ga stainless injector needle inserted 1 mm beyond the tip of the implanted guide tube. A Harvard syringe pump (1.0 μl/min) with a Hamilton 10 μl syringe delivered 0.5 μl of the drug solution over 30 seconds. The injector remained in place for 30 seconds longer to ensure complete diffusion of the drug solution. All solutions were kept at 37°C by a water bath. Injections were carried out between 10:00 and 11:00 a.m. All the animals were acclimatized to the injector-recording procedure 3 days before the start of the experiments, and were given a typical injection (0.5 μl) of saline to clear the injection area. Brain temperature (T_{br}) was recorded 30 min before injection, and then every 10 min for 3 h. EEG was recorded for 6 h. Sleep latencies and first h of sleep were determined from the moment after each rat was injected. However, 0–3-h, 3–6-h, and 0–6-h sleep assessments in all rats were measured 15 minutes after the last rat was injected. Eight rats were injected and polygraphically recorded

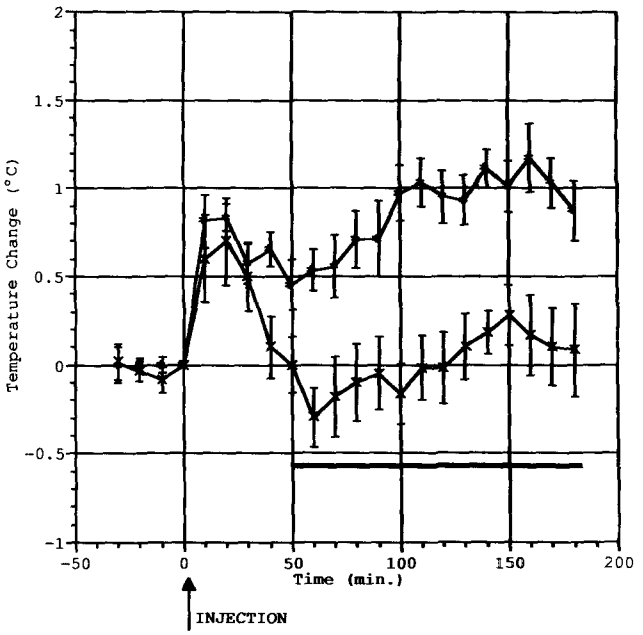

FIG. 2. The effects of handling of rats without saline injection compared to handling of rats with saline injection on brain temperature. —:$p<0.05$: Significantly different from handling of rats with saline injection. *: Handling with saline injection (n=6), X: Handling without saline injection (n=6).

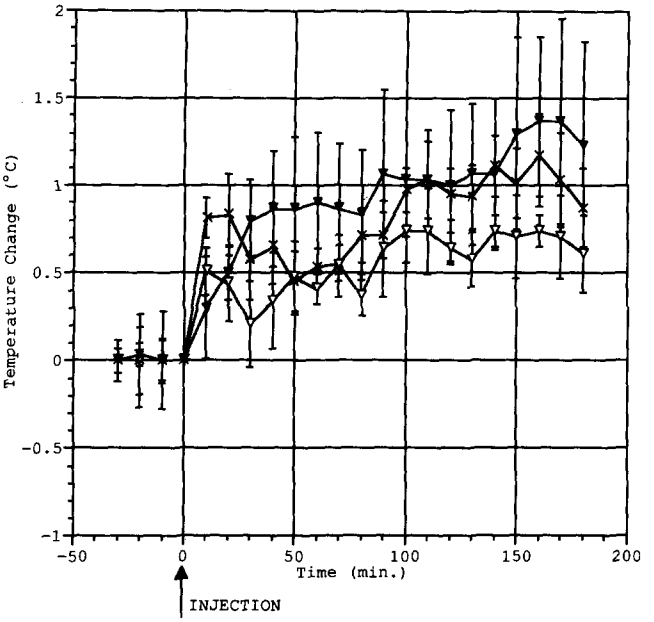

FIG. 3. Dose-response effects of adenosine on brain temperature in rats. X: Saline (n=7), ▽: 12.5 nmoles adenosine (n=6), ▼: 25.0 nmoles adenosine (n=3).

during an experiment. However, to minimize environmental and sensitization factors, the rats were split into two groups of four with each group receiving a different sequence of drug injections and neither group receiving the same drug treatment on the same day. Gross behavior during all recording sessions was continuously monitored using a video camera and remote monitor. Analysis of the EEG data was identical to that described in earlier reports (47) using an automated system of sleep state analysis which analyzes the sleep-wake states for as many as eight rats at a time (6). The system is based on a determination of threshold values for each parameter for each rat, then finding the natural transitions from one sleep state to another. These states were then classified utilizing the cortical EEG and EMG signals. Polygraphic recordings were assessed by 1-min epochs and categorized to either wake, slow wave sleep-1 (SWS_1), SWS_2, or REM according to the predominant electrographic activity within that epoch. Wakefulness was defined as a high-frequency, low-amplitude EEG with concomitant high EMG tone. SWS_1 was defined by the appearance of spindles and less than 50% low-frequency, high-amplitude EEG delta slow waves, while SWS_2 was defined as greater than 50% or more delta slow waves. Finally, REM was defined by low-amplitude, high-frequency EEG with an absence of EMG tone.

Statistical Analysis

Polygraphic recordings were analyzed using one-way analysis of variance (ANOVA). When statistically significant ($p<0.05$) ANOVA F values were observed, multiple comparisons between treatment means were made using the least significant difference procedure (38). One-way analysis of variance was also used to compare T_{br} for the corresponding doses of each drug treatment against the vehicle control for every 10-minute interval in the three hours of temperature recording. Data comparing IN PO to

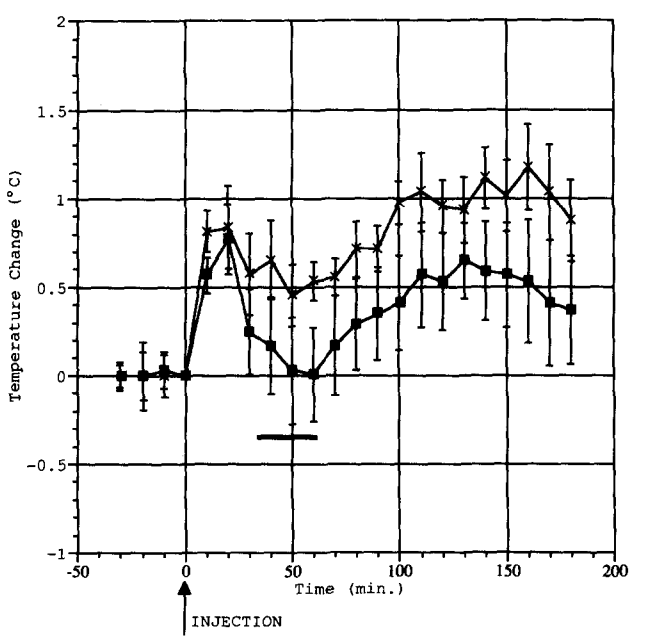

FIG. 4. The effects of NECA on brain temperature in rats. —: $p<0.05$: Significantly different from saline control. X: Saline (n=5), ■: 1.0 nmole NECA (n=5).

TABLE 1

DOSE-RESPONSE EFFECTS OF ADENOSINE ON SLEEP AND SLEEP LATENCIES IN RATS[1]

Sleep State	Recording Period (h)	Saline (n=15)	Adenosine (nmoles) 12.5 (n=12)	25.0 (n=5)
W	0–6	148 ± 7	125 ± 14*	147 ± 9
	1	12 ± 2	8 ± 2	15 ± 4
SWS_1	0–3	38 ± 5	32 ± 5	34 ± 4
	3–6	40 ± 4	33 ± 4	31 ± 5
	0–6	78 ± 8	65 ± 12	65 ± 7
	1	10 ± 2	18 ± 3*	10 ± 2
SWS_2	0–3	68 ± 6	85 ± 5*	79 ± 4
	3–6	47 ± 4	61 ± 5*	49 ± 7
	0–6	115 ± 9	146 ± 10*	128 ± 7
	1	5 ± 2	2 ± 1	2 ± 1
REM	0–3	10 ± 1	16 ± 2*	8 ± 2†
	3–6	10 ± 1	14 ± 2	12 ± 4
	0–6	20 ± 3	30 ± 2*	20 ± 5
	1	27 ± 2	28 ± 2	27 ± 4
Total Sleep	0–3	115 ± 4	133 ± 4*	120 ± 5
	3–6	97 ± 6	108 ± 6	92 ± 9
	0–6	212 ± 6	241 ± 10*	213 ± 9
Sleep Latencies				
SWS_1		19 ± 3	14 ± 2	23 ± 6
SWS_2		25 ± 3	24 ± 7	25 ± 5
REM		71 ± 27	53 ± 13	39 ± 9

[1]All values reported are means ± SEM (minutes) with numbers of rats shown in parentheses.

*$p<0.05$: Significantly different from saline control.

†$p<0.05$: Significantly different from 12.5 nmol adenosine group.

TABLE 2

DOSE-RESPONSE EFFECTS OF NECA ON SLEEP AND SLEEP LATENCIES IN RATS[1]

Sleep State	Recording Period (h)	Saline (n=7)	NECA (nmoles) 0.5 (n=7)	1.0 (n=6)
W	0–6	150 ± 11	120 ± 20	119 ± 18*
	1	12 ± 2	8 ± 2	13 ± 1
SWS_1	0–3	39 ± 7	49 ± 9	30 ± 4
	3–6	40 ± 7	41 ± 8	36 ± 4
	0–6	79 ± 45	90 ± 16	66 ± 4
	1	10 ± 2	1 ± 5	11 ± 5
SWS_2	0–3	65 ± 10	74 ± 5	90 ± 13*
	3–6	46 ± 4	61 ± 14	73 ± 8*
	0–6	111 ± 15	135 ± 10	163 ± 20*
	1	5 ± 2	1 ± 0	2 ± 0
REM	0–3	10 ± 2	5 ± 3	5 ± 2
	3–6	10 ± 2	11 ± 3	7 ± 1
	0–6	20 ± 3	15 ± 5	12 ± 2
	1	27 ± 2	17 ± 1	17 ± 1
Total Sleep	0–3	114 ± 7	127 ± 4	125 ± 15
	3–6	96 ± 6	113 ± 16	116 ± 11
	0–6	210 ± 11	240 ± 21	241 ± 18*
Sleep Latencies				
SWS_1		19 ± 3	31 ± 5	20 ± 1
SWS_2		25 ± 3	40 ± 11	25 ± 2
REM		71 ± 27	81 ± 36	141 ± 59

[1]All values reported are means ± SEM with numbers of rats shown in parentheses.

*$p<0.05$: Significantly different from saline.

OUT PO injections in Table 5 were analyzed using a 2-way ANOVA table.

Histology

At the end of the experiments, the location of the sites of microinjection was verified by means of standard histological procedures. After the animal was given an overdose of sodium pentobarbital, saline followed by 10% buffered neutral formalin was perfused through the aorta. The brain was removed, blocked and then frozen sections were cut on a cryotome at 40 μm and stained by cresyl violet. The position of each microinjection site was verified under light microscopy and mapped as represented in Fig. 1. Injection sites were differentiated into two groups: bilateral injections that had both sites within the PO (IN PO) and bilateral injections that had both sites adjacent to but not within the PO (OUT PO).

RESULTS

The effects on sleep and temperature of saline injection to the PO were investigated in three studies. First, a control study showed no effects on sleep after bilateral saline injection to the PO for any sleep stage or time interval (handling of rats without saline injection vs. handling of rats with saline injection: n=8 for each group, $p>0.05$; data not shown). While sleep characteristics were not altered, significant temperature effects with saline injection were seen (Fig. 2). After routine handling, the rats responded with an increase in T_{br} that lasted 30 minutes. In addition to this initial hyperthermia, saline injection produced an increase in temperature lasting from 50 to 180 minutes after the injection. Although this continued hyperthermia may be due to acute tissue damage (34), our data show no correlation between temperature changes and hypnotic effects.

Our second control study showed that repeated microinjections to the PO also had no effects on sleep. For example, no differences were found between administration of saline early in the study (1st or 2nd injection) and administration of saline late in the study (6th,7th, or 8th injection) (early injection, n=5; late injection, n=4; $p>0.05$; data not shown). In addition, the effect of adenosine on sleep was no different when adenosine was administered early or late in the study (12.5 nmoles adenosine: early injection, n=7; late injection, n=5; $p>0.05$: data not shown).

Our third control study compared the effects on sleep and temperature of bilateral saline injections to rats with both injectors in the PO (IN PO) versus bilateral saline injections in rats where both injectors were within 0.5 mm of the PO, yet still outside the PO (OUT PO) (see Fig. 1). There were no significant differences in sleep or in temperature effects between these two data sets (IN PO, n=18; OUT PO, n=8; $p>0.05$; data not shown).

A bilateral injection of adenosine (12.5 nmoles) to the PO caused a significant hypnotic effect (Table 1). An increase in total sleep for the 12.5 nmole injection of adenosine was observed in the 0–3- (16%), and 0–6-hour (14%) intervals. Increases in SWS_2 at this dose were 80% in the first h, 25% in

TABLE 3

DOSE-RESPONSE EFFECTS OF CPA ON SLEEP AND SLEEP LATENCIES IN RATS[1]

Sleep State	Recording Period (h)	CPA (nmoles) Saline (n=20)	0.25 (n=6)	0.5 (n=8)	1.0 (n=7)	6.25 (n=4)
W	0–6	133 ± 7	112 ± 10*	132 ± 9	129 ± 5	151 ± 10
SWS_1	1	6 ± 2	6 ± 2	8 ± 2	13 ± 1	13 ± 1
	0–3	34 ± 4	35 ± 5	26 ± 4	29 ± 4	39 ± 10
	3–6	36 ± 4	39 ± 5	1 ± 5	31 ± 4	47 ± 8
	0–6	70 ± 7	74 ± 9	57 ± 8	60 ± 7	86 ± 15
SWS_2	1	13 ± 2	7 ± 2	12 ± 4	10 ± 5	7 ± 4
	0–3	73 ± 6	81 ± 8	79 ± 7	80 ± 4	65 ± 9
	3–6	55 ± 5	65 ± 4	66 ± 5	64 ± 5	49 ± 11
	0–6	128 ± 10	146 ± 13*	146 ± 7*	145 ± 4*	114 ± 16†
REM	1	2 ± 2	1 ± 0	1 ± 0	2 ± 0	3 ± 1
	0–3	12 ± 2	12 ± 3	14 ± 3	13 ± 2	2 ± 3†
	3–6	10 ± 2	16 ± 3	11 ± 2	14 ± 2	5 ± 2†
	0–6	22 ± 3	28 ± 5	25 ± 4	27 ± 4	8 ± 4*†
Total Sleep						
	1	21 ± 2	14 ± 3	20 ± 5	17 ± 7	13 ± 7
	0–3	119 ± 7	128 ± 7	120 ± 8	122 ± 7	105 ± 11
	3–6	101 ± 6	120 ± 6	108 ± 9	109 ± 6	103 ± 14
	0–6	220 ± 11	248 ± 11*	228 ± 9	231 ± 5	209 ± 10†
Sleep Latencies						
SWS_1		19 ± 3	38 ± 9	43 ± 1	31 ± 5	36 ± 12
SWS_2		25 ± 3	49 ± 5	46 ± 11	37 ± 7	38 ± 12
REM		71 ± 27	88 ± 34	81 ± 36	73 ± 10	208 ± 65*†

[1]All values reported are means ± SEM with numbers of rats shown in parentheses. *$p<0.05$: Significantly different from saline. †$p<0.05$: Significantly different from 0.5 and 1.0 nmole CPA groups.

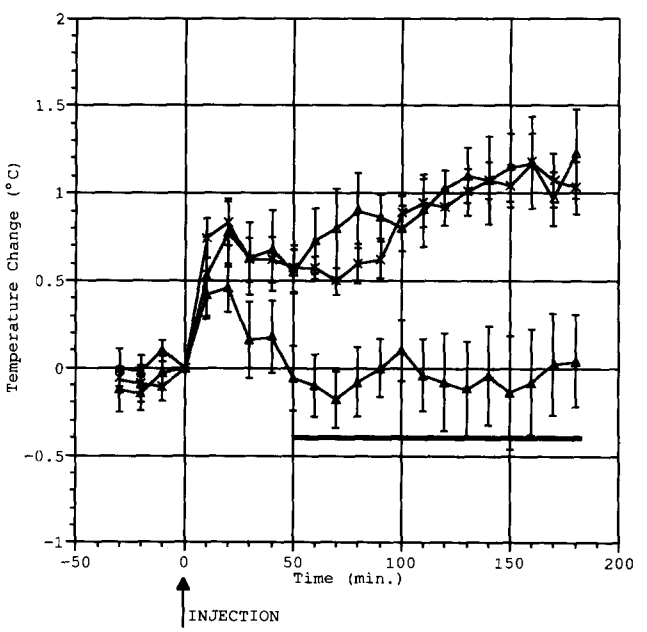

FIG. 5. Dose-response effects of CPA on brain temperature in rats. —: $p<0.05$: Significantly different from saline control. X: Saline (n=7), △: 0.25 nmoles CPA (n=4), ▲: 0.5 nmoles CPA (n=5).

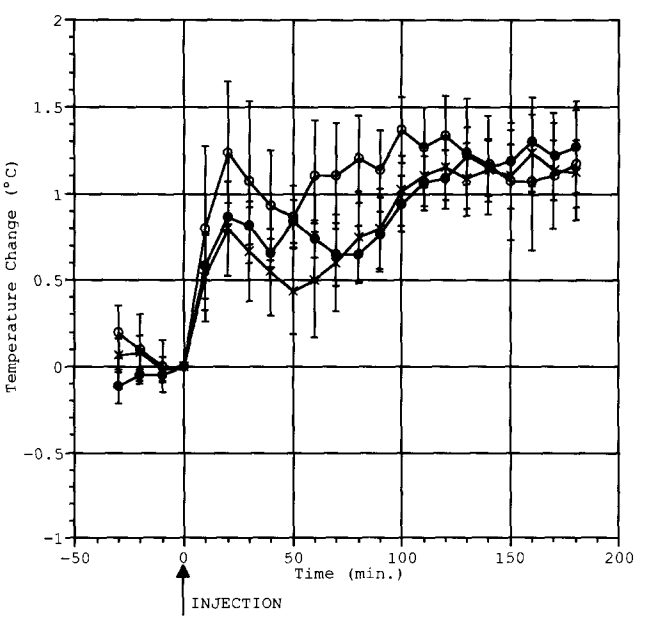

FIG. 6. Dose-response effects of CV-1808 on brain temperature in rats. X: Saline (n=7), ○: 5 nmoles CV-1808 (n=5), ●: 10 nmoles CV-1808 (n=6).

TABLE 4

DOSE-RESPONSE EFFECTS OF CV-1808 ON SLEEP AND SLEEP LATENCIES IN RATS[1]

Sleep State	Recording Period (h)	Vehicle (n=8)	CV-1808 (nmoles) 5.0 (n=8)	10.0 (n=7)
W	0–6	139 ± 13	104 ± 9	109 ± 13
	1	8 ± 2	6 ± 1	4 ± 1
SWS_1	0–3	28 ± 3	35 ± 2	32 ± 5
	3–6	34 ± 4	34 ± 4	32 ± 4
	0–6	62 ± 6	69 ± 3	64 ± 7
	1	9 ± 3	20 ± 4	14 ± 4
SWS_2	0–3	80 ± 10	94 ± 6	91 ± 7
	3–6	62 ± 6	77 ± 5	72 ± 6
	0–6	142 ± 11	171 ± 8	163 ± 13
	1	2 ± 1	3 ± 1	2 ± 1
REM	0–3	9 ± 3	9 ± 3	12 ± 3
	3–6	8 ± 3	7 ± 2	12 ± 3
	0–6	17 ± 5	16 ± 4	24 ± 5
	1	19 ± 2	29 ± 5	20 ± 5
Total Sleep	0–3	117 ± 12	138 ± 7	135 ± 6
	3–6	104 ± 6	118 ± 7	116 ± 9
	0–6	221 ± 13	256 ± 13	251 ± 13
Sleep Latencies				
SWS_1		38 ± 8	27 ± 2	36 ± 6
SWS_2		41 ± 9	29 ± 7	40 ± 5
REM		71 ± 27	55 ± 13	53 ± 9

[1]All values reported are means ± SEM (minutes) with numbers of rats shown in parentheses.

TABLE 5

COMPARISON OF EFFECTS ON SLEEP DURING THE FIRST HOUR OF EEG RECORDING OF DRUG TREATMENTS INJECTED INTO THE PREOPTIC AREA (IN PO) AND AROUND THE PREOPTIC AREA (OUT PO)[1]

Drug	OUT PO	IN PO
Saline	(n=6)	(n=10)
SWS_2	7 ± 3	10 ± 2
Total Sleep	29 ± 6	26 ± 2
Adenosine (12.5 nmoles)	(n=6)	(n=10)
SWS_2	7 ± 3	18 ± 2*
Total Sleep	14 ± 6	28 ± 6
NECA (1.0 nmole)	(n=4)	(n=9)
SWS_2	11 ± 6	11 ± 4
Total Sleep	2 ± 2	17 ± 4
CPA (0.5 nmole)	(n=3)	(n=9)
SWS_2	12 ± 3	13 ± 2
Total Sleep	14 ± 6	12 ± 4
CV-1808 (10 nmoles)	(n=3)	(n=7)
SWS_2	13 ± 3	14 ± 4
Total Sleep	14 ± 6	20 ± 5

[1]All values reported are means ± S.E.M. with numbers of rats shown in parentheses.

*$F(3,29)=6.45$, $p<0.05$, significant drug × loci interaction.

the 0–3-h interval, 30% in the 3–6-h interval, and 27% in the 0–6-h interval. REM sleep increased during the 0–3-h (60%) and 0–6-h (50%) intervals. While adenosine increased slow-wave sleep in the first hour when injected to the PO, OUT PO injections of adenosine had no effect on sleep during the same time interval (Table 5). No changes in sleep latencies were observed when adenosine was injected either to the PO or OUT PO. Also, no differences in sleep effects between IN PO and OUT PO injections were found after the first hour following adenosine administration. In contrast to the 12.5 nmole dose, 25.0 nmoles adenosine did not produce a hypnotic effect. A decreased hypnotic effect with increasing doses was also observed with increasing doses of CPA in the present study and in previous studies when adenosine A1 agonists were injected peripherally (36). As shown in Fig. 3, neither the 12.5 nmole nor the 25.0 nmole dose of adenosine caused an effect on T_{br} as compared to saline.

Bilateral administration to the PO of NECA, a nonselective A1/A2 agonist (A1 $k_i=6.3$ nmol/l, A2 $k_i=10$ nmol/l, A2 k_i/A1 $k_i=1.6$) (5), produced increases in SWS_2 and total sleep (Table 2). At a dose of 1.0 nmole, NECA produced an increase in total sleep (15%) for the 0–6-h interval. At this dose, SWS_2 duration also increased during the 0–3- (38%), 3–6- (59%) and 0–6- (47%) hour intervals. No differences between IN PO and OUT PO injections were observed with 1.0 nmole NECA during any time interval analyzed (data not shown except for the first hour of polygraphic recording, see Table 5). Several rats receiving the bilateral injection of 1.0 nmole NECA displayed a synchronized EEG equivalent to SWS_2 while being awake and moving. This discrepancy between synchronization of the EEG and observed gross behavior did not occur with any other drug injection or a decreased dose of NECA whether or not the drug treatment increased sleep. Therefore, these recordings were excluded from the data. The 1.0 nmole dose of NECA caused a significant change in T_{br} as compared to saline lasting from 40 to 70 minutes after injection (Fig. 4).

Bilateral administration to the PO of CPA (0.25, 0.5, and 1.0 nmoles), a selective adenosine A1 receptor agonist (A1 $k_i=0.59$ nmol/l, A2 $k_i=460$ nmol/l, A2 k_i/A1 $k_i=0.001$) (5), produced increases in SWS_2 and/or total sleep (Table 3). CPA increased SWS_2 during the 0–6-h interval at doses of 0.25 (14%), 0.5 (14%) and 1.0 (14%) nmoles in comparison to saline. In addition, the 0.25 nmole dose of CPA increased total sleep (13%) during the 0–6-h interval. No differences between IN PO and OUT PO injections were observed with 0.25 nmoles CPA during any time interval analyzed (data not shown except for the first hour of polygraphic recording, see Table 5). The highest dose of CPA (6.25 nmoles) produced both a decrease in REM sleep and an increase in REM sleep latency as compared to saline controls (Table 3). Initial injections of the 12.5 nmole dose of CPA caused EEG seizures in rats without producing convulsions. While the 0.25 nmole dose of CPA caused no changes in T_{br} as compared to saline, the 0.5 nmole dose of CPA caused a significant hypothermia lasting from 50 minutes after injection until the end of the temperature recording period at 180 minutes (Fig. 5).

CV-1808 (5 and 10 nmoles), a selective adenosine A2 receptor agonist (A1 $k_i=560$ nmol/l, A2 $k_i=120$ nmol/l, A2 k_i/A1 $k_i=4.67$) (5), when injected bilaterally to the PO, produced no effect on sleep (Table 4). Likewise, bilateral injection of both doses of CV-1808 had no effect on T_{br} as compared to vehicle injection (Fig. 6). These doses of CV-1808 (5 and 10 nmoles) were selected because they were previously shown to have hypnotic effects when microinjected to the striatum of rats (manuscript in preparation).

DISCUSSION

The present study is in agreement with previous findings

showing the central hypnotic effect of adenosine in rats (36). Specifically, the data indicate a functional role of adenosine A1 receptors in sleep regulation within the preoptic area, since bilateral microinjection to the PO of adenosine, NECA, and the selective adenosine A1 agonist, CPA, increased sleep. In support for this role of adenosine A1 receptors are recent findings suggesting circadian and ultradian rhythms in cAMP concentration in the PO. Thus, during the 12-h light period, when rats sleep 2.5 h more than in the 12-h dark period (12), cAMP concentration in the PO decreased (49). In addition, cAMP concentration in the PO was lower during SWS_2 and REM sleep as compared to wakefulness (49). Since activation of adenosine A1 receptors inhibits adenylate cyclase (44), thereby reducing cAMP concentration, it is possible that stimulation of the adenosine A1 receptors by adenosine, NECA, and CPA might have decreased cAMP in the PO leading to increased sleep. In accordance, a previous study has shown that binding of the A1 receptor agonist [^{3}H]CHA actually increases in the hypothalamus of rats exposed to various stress models that cause sleep disruption (1).

In contrast to the other drug treatments, the A2 specific adenosine receptor agonist, CV-1808, had no significant effect on sleep when microinjected to the PO, which agrees with autoradiographic studies indicating that adenosine A2 receptors are absent from the hypothalamus (17). However, CV-1808 did have a tendency to increase SWS_2 and total sleep during the 6-h EEG recording. Since CV-1808 can potently block cellular adenosine transport in addition to stimulating the adenosine A2 receptor, the tendency for increased sleep in the present study might have been due to stimulation of the adenosine A1 receptors by an increased extracellular adenosine concentration (2). Therefore, the lack of a relative contribution of adenosine A2 receptor stimulation on sleep cannot be completely ascertained with CV-1808. However, the same doses of CV-1808 which had no significant effect on sleep in the present study, showed a hypnotic effect when microinjected to the striatum, a brain area reported to possess a high density of adenosine A2 receptors (manuscript in preparation) (17).

To assess whether the hypnotic effects of the drug treatments originated in the PO, we compared bilateral injections within the PO (IN PO) to those adjacent to the PO (OUT PO). All the bilateral injections outside the PO were localized to points within 0.5 mm of the preoptic area circumference. Despite this close proximity to the preoptic area of OUT PO injections, adenosine administration at these locations failed to increase SWS_2 during the first hour. In contrast, SWS_2 increased within the first hour following adenosine microinjection within the PO. A previous study of intracerebral injections to rats showed that injected volumes of 0.5 μl diffuse 0.3–0.8 mm from the center of the injection site (24). The ability of microinjected drugs to diffuse into the PO from OUT PO loci might therefore account for the similar hypnotic effects in these two groups of rats after the first hour of EEG recording. NECA, a polar compound, has been shown to diffuse both longer and further in the CNS than more lipophilic adenosine analogs making its precise site of action tenuous (13). However, although possible, it is generally not supported that a substance injected into the hypothalamus could exert its effect on a remote structure by a ventricular route (24–26).

Our data show that the effect on sleep of adenosine, NECA, and CPA was primarily that of sleep maintenance rather than sleep induction, which was indicated by increases in total sleep without a decrease in sleep latencies. In a similar manner, microinjections to the PO of triazolam, a benzodiazepine which potentiates adenosine-evoked depression of cerebral cortical neuronal firing (31), increased sleep duration over an 8-hour period, but failed to reduce sleep latencies (21).

Previous studies suggest that adenosine does not affect temperature regulation in the PO, although the PO is considered to be the major site for thermoregulatory control in the rat (4). For example, maximal doses of adenosine (2 mM) when injected unilaterally to the PO, failed to alter core temperature (7). In addition, injections to the PO of a nonselective adenosine receptor antagonist, aminophylline, and a selective adenosine A1 receptor antagonist, cyclopentyltheophylline, failed to affect temperature in rats exposed to ambient temperatures of $-10°C$ (47). In accordance, present results indicate that both doses of adenosine and the 0.25 nmole dose of CPA bilaterally injected to the PO did not affect T_{br}. However, under the same experimental conditions, 1.0 nmole NECA transiently decreased T_{br} during the first hour, while 0.5 nmoles CPA produced prolonged hypothermia. It is of interest that all doses of drugs that produced hypothermia increased SWS_2 and/or total sleep. The lack of correlation between sedative and temperature effects has already been reported in rats when adenosine analogs were administered peripherally (9).

In conclusion, this study demonstrates a site for the central hypnotic action of adenosine. The data show that administration of adenosine to the preoptic area of rats increased sleep, and that this effect appears to be due to stimulation of adenosine A1 receptors. Administration of adenosine to the PO apparently had no effect on sleep latencies or thermoregulation.

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