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Behavioral and Electroencephalographic Effects of the Adenosine$_1$ Agonist, L-PIA

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MARTIN, J. V., K. F. BERMAN, P. SKOLNICK AND W. B. MENDELSON. *Behavioral and electroencephalographic effects of the adenosine$_1$ agonist, L-PIA*. PHARMACOL BIOCHEM BEHAV **34**(3) 507–510, 1989.—The effects of N^6-(L-2-phenylisopropyl)-adenosine (L-PIA), an A_1 agonist, were measured on both spontaneous locomotor activity and electroencephalographic (EEG) measures of sleep in rats. L-PIA strongly inhibited motor activity at 100 μg/kg intraperitoneally (IP), a dose which had no statistically significant effects on EEG-defined sleep. A higher dose of L-PIA (200 μg/kg) increased the latency to sleep initiation and inhibited later REM sleep. These results demonstrate that L-PIA can produce a state of apparent behavioral quiescence in the presence of EEG-defined arousal.

N^6-(L-2-phenylisopropyl)-adenosine (L-PIA) Sleep REM Adenosine agonist Sedation Locomotor activity Electroencephalogram

BEHAVIORAL stimulants such as caffeine and theophylline have been demonstrated to competitively inhibit the effects of adenosine on adenylate cyclase activity in brain (19). This finding stimulated studies on the role of adenosine in the regulation of neural activity (5). Although the role of adenosine as neurotransmitter or neuromodulator is uncertain (23), it is clear that this nucleoside is present in relatively high concentrations in neural tissue, and has a variety of inhibitory effects on synaptic transmission (5). Based on pharmacological studies, extracellular receptors for adenosine have been classified as A_1 and A_2 (9,24). Occupation of A_1 receptors leads to an inhibition of adenylate cyclase activity whereas occupation of A_2 receptors mediates an increase in the activity of this enzyme (9,24). N^6-(L-2-phenylisopropyl)-adenosine (L-PIA), an adenosine analog, has been found to selectively bind to the A_1 receptor (20). This compound is resistant to degradation by adenosine deaminase and to phosphorylation (22), and has been used extensively to characterize the physiological effects mediated through the A_1 receptor site (14).

Adenosine analogs have been reported to elicit a number of behavioral effects, including anticonvulsant activity (2,6) and an inhibition of spontaneous locomotion (4, 6, 8). However, the role of adenosine in the regulation of sleep is less well-defined. A hypnotic effect of adenosine was reported in dogs (7), and L-PIA was reported to increase deep slow-wave sleep in rats without altering the values for total sleep and wakefulness (15). At somewhat higher doses (0.35 mg/kg) L-PIA was found to increase waking time at the expense of sleep (17). Similarly, inhibition of brain adenosine deaminase activity by deoxycoformycin (DCF) altered the proportion of nonREM sleep characterized by increased slow wave activity (16), while a second inhibitor, erythro-9-(2-hydroxy-3-nonyl)-adenine (EHNA), had no effect on total nonREM sleep (13). These findings indicate that increasing levels of endogenous adenosine may alter the structure of sleep with little effect on total sleep duration. Thus, administration of adenosine analogs or increasing brain concentrations of adenosine appears to have strong sedative effects as measured by locomotor behavior, but only subtle hypnotic effects with regard to electroencephalographic (EEG) measures of consciousness.

In order to examine effects of A_1 receptor agonists on the relationship between behavioral quiescence and sleep regulation, we have examined the effects of L-PIA on both EEG and locomotor behavior.

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METHOD

Animals

Male 200–250 g Sprague-Dawley rats were obtained from Taconic Farms (Germantown, NY). The rats were housed in groups of 3–5 (and after surgery, individually) in plastic tubs having solid bottoms lined with cedar bedding. Temperature was maintained at 25.0 to 28.3°C with lights on from 8:00 a.m. to 8:00 p.m. Food and water were available ad lib.

Drug Injections

N^6-(L-2-phenylisopropyl)-adenosine (Boehringer, Mannheim, West Germany) was dissolved in phosphate buffered saline (PBS; 154 mM NaCl, 5.6 mM Na_2HPO_4, 1.0 mM KH_2PO_4, pH 7.2) just prior to use. The drug (or vehicle) was administered intraperitoneally (IP) in a final volume of 1 ml/kg.

Locomotor Activity

The spontaneous locomotor activity of eight rats was measured in a lighted room using a Motron Produkter apparatus, which measures the number of interruptions of five horizontal light beams located 8 cm above a 22 × 34 cm field. At approximately 9:00 a.m. of the morning of a test, a rat was injected IP with either vehicle (PBS) or 100 μg/kg L-PIA, and placed in the testing chamber. Activity was measured over successive 10-min intervals for a 3-hr period. On a second occasion, at least one week later, the rat was given the alternative injection of either vehicle or 100 μg/kg L-PIA, and retested. Data were analyzed by a two-way analysis of variance (ANOVA) for repeated measures, examining effects of drug treatment and time.

Sleep Studies

For each of three treatment conditions (vehicle, 100 μg/kg L-PIA, or 200 μg/kg L-PIA) groups of 9–11 rats were implanted with electrodes. Prior to surgery, rats were anesthetized with an intramuscular injection of 70 mg/kg ketamine and 6 mg/kg xylazine. Four 0-80 stainless steel machine screws were implanted to act as dural electroencephalographic (EEG) electrodes, and two 0.010-inch Teflon-coated stainless steel wires were inserted into the nuchal musculature for electromyographic (EMG) recording. Further lengths of the stainless steel wire connected the electrodes to a connector plug (Amphenol Corp., Salem, NH) and the entire assembly was cemented in place using dental acrylic (Kerr Corp., Romulus, MI) (11). During a one-week recovery period the rats were accustomed to handling, and for the night preceding a study they were housed in the chambers in which they would be tested. The next morning, starting at approximately 9:00 a.m., each rat was given an IP injection of vehicle, 100 μg/kg L-PIA, or 200 μg/kg L-PIA, and an eight-hour sleep recording was immediately begun.

EEG and EMG were recorded on a Grass Model 78 polygraph (Grass Instrument Co., Quincy, MA) with a vertical calibration of 50 μV/cm and a paper speed of 10 mm/sec. The resulting records were scored by a single investigator who was unaware of the treatment condition. Each 30-sec epoch was designated as a) waking, b) nonrapid eye movement (REM) sleep or c) REM sleep according to standard criteria (11,12). To evaluate intra-rater reliability, five records were scored twice. Correlation coefficients for sleep latency and total sleep time were 0.996 and 0.985 respectively. Data were analyzed in four successive two-hour blocks by an analysis of variance (ANOVA) for one repeated measure (time) and one between-group measure (drug). Planned comparisons were made between the means of individual drug treatments and the mean of the corresponding control group for that time point.

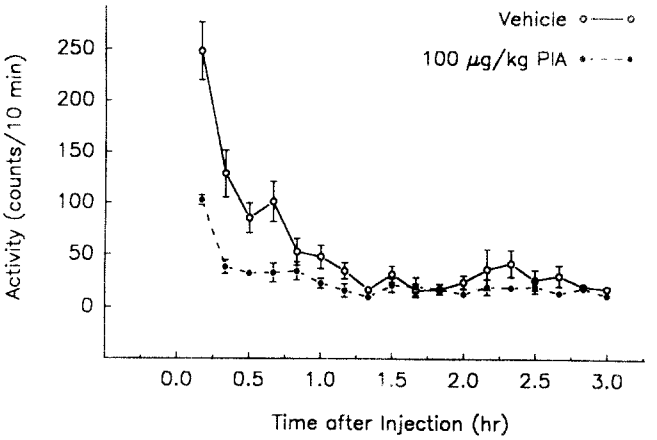

FIG. 1. Effect of L-PIA on spontaneous motor activity in rats. Rats were injected IP with 100 μg/kg L-PIA or an equivalent volume of PBS and activity was monitored by the number of interruptions of light beams in a testing chamber. Values are means ± SEM for 8 rats.

RESULTS

Locomotor Activity

As seen in Fig. 1, 100 μg/kg L-PIA caused a strong inhibition of spontaneous motor activity. The ANOVA indicated a highly significant effect of drug treatment, $F(1,7) = 36.5$, $p < 0.001$, and time, $F(17,119) = 27.3$, $p < 0.00001$. The effect of L-PIA was apparent mainly in the first hour of the three-hour test, reflected by a significant time period-by-drug treatment interaction, $F(17,119) = 9.3$, $p < 0.00001$. A separate ANOVA to examine the contribution of order of testing in vehicle-treated rats showed no significant effect, $F(1,6) = 5.0526$, $p > 0.05$.

Sleep Studies

A significant effect of dose of L-PIA, $F(2,26) = 3.98$, $p < 0.05$, was seen with respect to sleep latency, the time between the injection and the first 90 sec of uninterrupted sleep. As seen in Table 1, the sleep latency after an injection of 200 μg/kg of L-PIA was about 50% longer than the sleep latency measured in the group of rats injected only with vehicle. Similarly, the total time spent in sleep was significantly affected by the drug treatment, $F(2,26) = 3.23$, $p < 0.05$. Planned comparisons of means indicated that this effect was manifest in a decrease in total sleep time in the tests of the group injected with the high dose of L-PIA, as contrasted with the vehicle-injected control group (see Table 1). Accordingly, a significant time period-by-drug condition interaction was noted for total sleep, $F(6,78) = 2.22$, $p < 0.01$. A significant effect of drug treatment was also demonstrated with regard to REM sleep, $F(2,26) = 3.76$, $p < 0.05$. This effect on REM sleep may have contributed to the significant effect of drug treatment at the last part of the test, as a planned comparison showed a significant difference between the groups of rats treated with vehicle and 200 μg/kg L-PIA at 6–8 hours (see Table 1). Visual observation of the rats during the first two-hour period after injection confirmed that, as compared to vehicle treatment, both 100 μg/kg and 200 μg/kg of L-PIA inhibited spontaneous movement to a similar extent.

A significant effect of time was demonstrated in the ANOVA for each EEG parameter measured, e.g., total sleep, $F(3,78) = 8.99$, $p < 0.0001$. These effects represented well-known circadian

TABLE 1

EFFECTS OF 0.1 AND 0.2 mg/kg PIA ON SLEEP PARAMETERS OVER AN EIGHT-HOUR PERIOD IN RATS*

	Sleep Latency†	REM Latency‡	NonREM Sleep	Intermittent Waking Time§	REM Sleep	Total Sleep
0–2 Hours						
Vehicle¶	29.9 ± 6.5	156.3 ± 30.8	57.8 ± 5.5	31.1 ± 5.7	1.1 ± 0.7	58.9 ± 6.0
0.1 mg/kg PIA#	23.2 ± 2.7	160.8 ± 24.6	58.4 ± 5.0	37.6 ± 3.8	0.6 ± 0.3	59.0 ± 5.0
0.2 mg/kg PIA**	44.6 ± 7.2	221.5 ± 35.4	36.4 ± 6.0	38.0 ± 5.4	1.0 ± 0.9	37.4 ± 6.2††
2–4 Hours						
Vehicle			63.0 ± 5.1	56.3 ± 4.8	3.8 ± 1.1	63.0 ± 5.1
0.1 mg/kg PIA			56.4 ± 3.5	57.5 ± 3.1	4.3 ± 1.4	60.8 ± 3.2
0.2 mg/kg PIA			65.0 ± 2.9	53.9 ± 2.8	0.6 ± 0.4	65.6 ± 3.0
4–6 Hours						
Vehicle			58.3 ± 4.6	50.5 ± 5.5	10.7 ± 2.2	68.9 ± 5.7
0.1 mg/kg PIA			62.7 ± 4.8	46.2 ± 4.5	10.2 ± 1.1	72.9 ± 4.6
0.2 mg/kg PIA			54.5 ± 4.5	57.5 ± 4.7	5.2 ± 1.3	59.7 ± 4.3
6–8 Hours						
Vehicle			60.6 ± 4.0	44.8 ± 6.0	13.3 ± 2.6	73.9 ± 6.0
0.1 mg/kg PIA			56.6 ± 3.6	51.0 ± 4.2	11.4 ± 1.3	68.0 ± 4.2
0.2 mg/kg PIA			52.1 ± 3.6	59.7 ± 3.1	8.0 ± 1.3††	60.2 ± 3.1††

*Polygraph tracings were scored in 30-sec epochs for eight hours after the injection of drug or vehicle. Results are presented as the mean number of minutes ± S.E.M. of each EEG-defined stage of consciousness for four consecutive two-hour intervals. Sleep parameters are defined in detail in (10).

†Sleep latency was defined as the time from the injection until the first 90 uninterrupted seconds of sleep (sleep onset).

‡REM latency was defined as the time after the sleep onset until the occurrence of 60 seconds of REM sleep, interrupted by no more than 60 seconds of another stage of consciousness.

§Intermittent waking time was defined as the time scored as waking after the sleep onset.

¶n = 9.

#n = 11.

**n = 9.

††$p<0.05$ contrasted to Vehicle group in planned comparison.

effects on stage of consciousness (10).

DISCUSSION

The present experiments demonstrated a strong sedative effect of 100 μg/kg L-PIA with regard to spontaneous motor activity. No hypnotic effect of this dose of L-PIA was noted in parallel studies of EEG. Moreover, EEG-defined sleep was significantly inhibited by a higher dose (200 μg/kg) of the drug. The initiation of sleep, as measured by sleep latency, was significantly affected by L-PIA. Also, at later times (when REM sleep is normally highest, see Table 1), REM sleep was strongly inhibited by the higher dose of L-PIA. With respect to EEG measures, then, L-PIA had solely an arousing effect that was most evident at 200 μg/kg.

These results on EEG measures of sleep are generally consistent with previous reports regarding the effects of manipulations which increase the occupation of A_1 receptors by agonists. As mentioned before, inhibiting adenosine deaminase (which would presumably increase endogenous adenosine levels) causes dramatic decreases in motor locomotion (13), but only changes the relative proportions of the stages of sleep (16). Similarly, previous studies have demonstrated the inhibition of motor activity by relatively low doses (100 μg/kg) of L-PIA (6,8) and other adenosine analogs (4) in mice. Behavioral quiescence was also noted in rats at a somewhat higher dose of 2 mg/kg L-PIA by gross behavioral observation (6). With regard to EEG-defined stage of consciousness in rats, 12–115 μg/kg L-PIA changed a measure of the depth of slow wave sleep (15,17), but did not affect the absolute amount of time spent sleeping (17). In agreement with the present data, a somewhat higher dose of L-PIA (350 μg/kg) caused a wakening effect and decreased REM sleep (17). Therefore, although we did not distinguish between subtypes of non-REM sleep in the present study, the effects measured are consistent with previous reports of behavioral quiescence and EEG effects following administration of A_1 receptor agonists.

L-PIA has been reported to possess hypotensive actions (1,3). The cardiovascular effects of adenosine are mediated, in part, by a direct action on peripheral tissues (3). While adenosine does not appear to cross the blood-brain barrier in appreciable quantities (3), peripherally injected doses of L-PIA comparable to those used in the present study can accumulate in brain at concentrations consistent with an action at central A_1 receptors (8). In addition, the behavioral (and some hypotensive) effects of L-PIA can be elicited by central injection of the compound (1). The threshold doses for the hypotensive effects of systemic L-PIA are consistently reported to be 10–100-fold higher than those required for effects on locomotor activity (8,5). It is therefore unlikely that the effects of the doses of L-PIA used in the present study can be attributed to a peripheral cardiovascular action of the drug.

On the other hand, the hypothermic activity of L-PIA does occur in the range of doses used in the present investigation (6). However, manipulations that induce hypothermia generally increase sleep (21). Although the potential role of thermoregulation in the present investigation was not addressed, it is of interest that doses of L-PIA which should have hypothermic effects (6) inhibited both sleep and spontaneous locomotor activity. L-PIA

may therefore serve as an important pharmacological tool for investigating the relationship between thermoregulatory mechanisms and sleep.

The present findings support the apparent dichotomy observed between previously reported effects of adenosine analogs on spontaneous locomotor activity and on EEG-defined sleep. The dissimilarities between the results of these earlier studies, then, probably did not reflect differences of species or routes of drug administration, but, rather, a distinction between the underlying mechanisms for behavioral quiescence and sleep. Increasingly, the need for careful differentiation between behavioral quiescence and sleep is being brought out (13), particularly with regard to the function of adenosine in brain (18). Our findings are consistent with a role of adenosine in inducing a state of behaviorally quiet arousal.

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