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p-SPA, a Peripheral Adenosine A_1 Analogue, Reduces Sleep Apneas in Rats

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MONTI, D., D. W. CARLEY AND M. RADULOVACKI. p-*SPA, a peripheral adenosine A_1 analogue, reduces sleep apneas in rats.* PHARMACOL BIOCHEM BEHAV **53**(2) 341-345, 1996.—The actions of N^6-*p*-sulfophenyladenosine (*p*-SPA), a novel peripherally selective adenosine A_1 agonist, were assessed on spontaneous and postsigh central sleep apneas in freely moving, unanesthetized rats by simultaneously monitoring sleep and respiration. Intraperitoneal administration of 0.1, 0.3, and 1.0 mg/kg of the drug significantly decreased postsigh and spontaneous sleep apnea index (AI). This effect persisted throughout the 6-h recording period. Doses of 0.1 and 0.3 mg/kg did not affect sleep efficiency, whereas 1.0 mg/kg of *p*-SPA reduced it to 60% of baseline value.

N^6-*p*-Sulfophenyladenosine (*p*-SPA) Sleep apneas Rats Adenosine A_1 receptors Peripheral chemoreceptors

SLEEP apnea syndrome is an important public health concern with a prevalence of approximately 2-8% of the adult population worldwide (1,13,15). A recent survey showed that 9% of women and 24% of men had more than five apneic events and that the prevalence of patients who met the diagnostic criteria of sleep apnea syndrome (five or more apneas-hypopneas and excessive daytime sleepiness) was 4% and 2% of men and women, respectively (20). Classically, these events are characterized as central, obstructive, or mixed, according to their pathophysiological characteristics.

The occurrence of centrally generated apneic events has been reported in rats of different strains, and several authors have previously referred to these events as models for understanding the basis of sleep-related breathing disorders (10,11, 17,19). We have previously documented that $R(-)N^6$-L-(2-phenylisopropyl)adenosine (L-PIA) and 2-*p*-(2-carboxyethyl)-phenethylamino-5′-*N*-ethylcarboxamidoadenosine hydrochloride (CGS 21680), A_1 and A_2 adenosine agonists, respectively, decreased the expression of apneic events during sleep in rats and hypothesized this action to be mediated through peripheral chemoreceptor organs (11).

In an effort to further evaluate the peripherally mediated role of adenosinergic modulation of respiratory control during sleep, we examined the effects of N^6-*p*-sulfophenyladenosine (*p*-SPA), a novel, water-soluble, and selectively peripheral adenosine A_1 receptor agonist (6), on the incidence of central sleep apneas in Sprague-Dawley rats.

METHOD

Eleven Sprague-Dawley adult rats (250-450 g) were anesthetized using a mixture of ketamine (80 mg/kg) and acetylpromazine (2 mg/kg) administered by IM injection followed by surgical incision of the scalp, bilateral implantation of stainless steel screws into the frontal and parietal bones of the skull for electroencephalographic (EEG) recording, and bilateral implantation of wire electrodes into the dorsal nuchal muscles for electromyographic (EMG) recording. All electrode leads were soldered to a miniature connector plug and fixed to the skull with dental cement. Following implantation the wound was closed with sutures.

Respiration was recorded by placing each rat, unrestrained, inside a single chamber body plethysmograph (PLYUN1R/U; Buxco Electronics, Sharon, CT; dimensions 6″ W × 10″ L × 6″ H) ventilated with a flow of fresh room air at a rate of 2 l/min. A cable plugged onto the animal's connector and passed through a sealed port to the polygraph was used to carry through bioelectric activity from the head. Both respira-

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tion and sleep-wake activities were recorded simultaneously on a Grass Model 79D polygraph at a paper speed of 5 mm/s.

Seven to 10 days were allowed for recovery after surgery. The day before the recording session each animal was housed for 16 h in the respiratory chamber to become habituated. Throughout the study, all animals were maintained in a fixed environment at 20°C, with 40% humidity, and cycled for 12 L : 12 D. Polygraphic recordings were made from 1000 to 1600 h and the administration of the vehicle or the adenosine agonist was done 15 min before starting to record (11,16).

Rats were given either vehicle (saline) or 0.1, 0.3, or 1.0 mg/kg of *p*-SPA. Prior to recording, rats were treated by IM injection with either vehicle or one of the doses. *p*-SPA was dissolved in saline solution previous to administration and concentrations were adjusted for bolus injection of 1 ml/kg. The study was designed in a repeated-measures pattern; each animal received all treatments assigned in random order and separated by at least 3 days.

Polygraphic recordings of sleep and wake were assessed using bifrontal and fronto-occipital EEG and nuchal EMG signals as guidelines on single 60-s epochs. Wakefulness (W) was defined as a high-frequency, low-amplitude EEG with concomitant high EMG tone. Non-slow wave (NSW) was defined by the appearance of spindles and less than 50% high-amplitude, low-frequency EEG delta slow waves, whereas slow-wave sleep (SWS) was defined as having 50% or more delta slow waves. We defined non-REM sleep as the sum of NSW and SWS (NSW + SWS). Rapid eye movement sleep (REM) was characterized by a high-frequency, low-amplitude EEG and an absence of EMG tone. Sleep efficiency was measured for each study as percentage of total epochs staged as sleep [((NSW min + SWS min + REM min)/360) × 100].

Sleep apneas, defined as cessations of respiratory effort for at least 2.5 seconds, were scored for each recording session and were associated with the stage in which they occurred: W, NSW, SWS, or REM sleep. The duration requirement of 2.5 s was arbitrarily chosen following analysis of pilot data from seven rats not included in the present study. The mean interbreath interval over all sleep stages was 0.8 ± 0.23 (SD) s; 2.5 s thus represents approximately 2 "missed" breaths. The events detected represent central apneas, because decreased ventilation associated with obstructed or occluded airways would generate an increased plethysmographic signal, rather than a pause. Similar to other authors (10,19), apneas were either observed to occur as pauses between breaths (spontaneous) or as periods of respiratory cessation preceded in general by a sigh (postsigh). We therefore characterized them as postsigh and spontaneous according to the presence or absence of a preceding inspiration at least 250% larger than the average amplitude during regular breathing (19) (Fig. 1). Apnea indexes (AI), defined as apneas per hour in stage were separately determined for NSW, SWS, and REM for every study. The specific effects of *p*-SPA on the AI were assessed by analyzing data pooled from the total recording time, and time course of

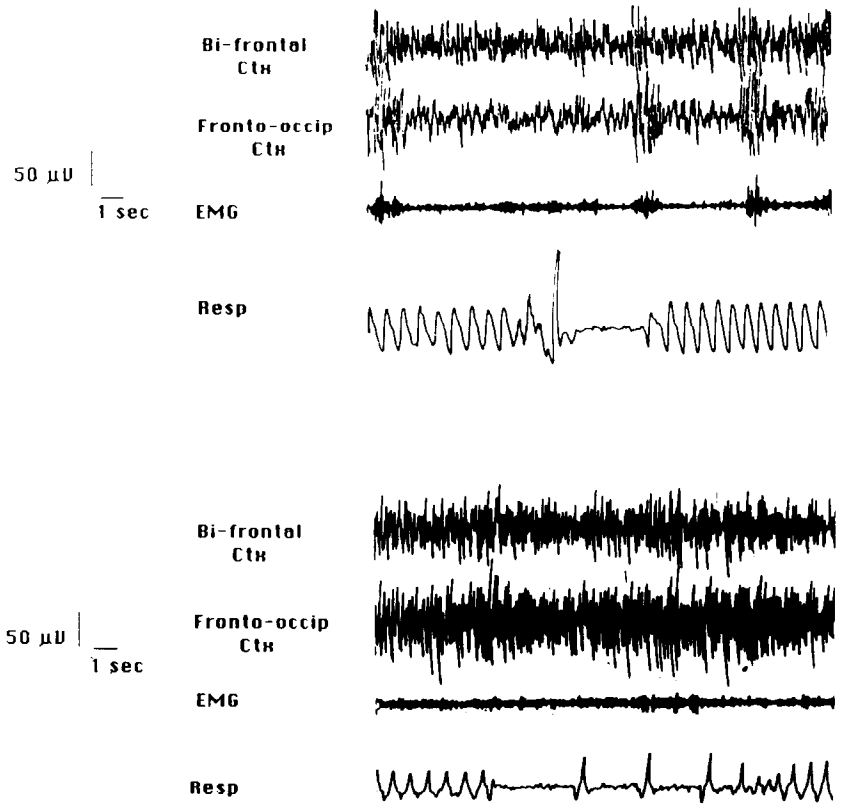

FIG. 1. Characterization of postsigh (upper) and spontaneous (lower) apneas recorded during sleep. Bi-frontal Ctx = EEG bi-frontal cortex; fronto-occip Ctx = EEG fronto-occipital cortex; EMG = electromyogram; Resp = respiratory activity. See text for explanation.

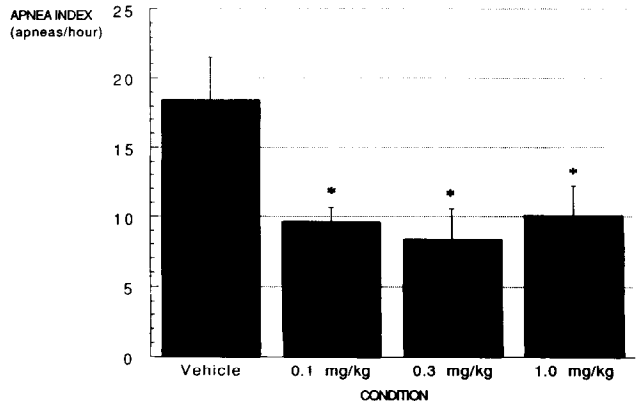

FIG. 2. Effects of 0.1, 0.3, and 1.0 mg/kg of *p*-SPA on the incidence of sleep AI during 6 h. All doses significantly reduced AI compared to vehicle administration (*$p < 0.05$ related to vehicle).

p-SPA effect was observed by separately examining AI on each third of the total recording time. One-way analysis of variance (ANOVA) was employed to evaluate the effects of dose, time-in-recording, and sleep architecture on AIs. Interaction effects were evaluated using multi-way ANOVA. Multiple comparisons between means were evaluated using Fisher's protected least significant difference (PLSD) (18). Results from one rat, whose pattern of response dramatically deviated from the rest, were left out of the statistical analysis to not invalidate the use of the parametric test.

RESULTS

Effects of p-*SPA on Sleep Apneas*

All doses of *p*-SPA significantly reduced postsigh AI to approximately 50% compared to baseline during total sleep time (Figs. 2, 3); both the 0.3 and 1.0 mg/kg doses evoked changes in spontaneous apneas ($p < 0.05$) (Fig. 3). At all doses the effects were specific to non-REM sleep (Fig. 4); no changes were observed in apneas related to REM sleep.

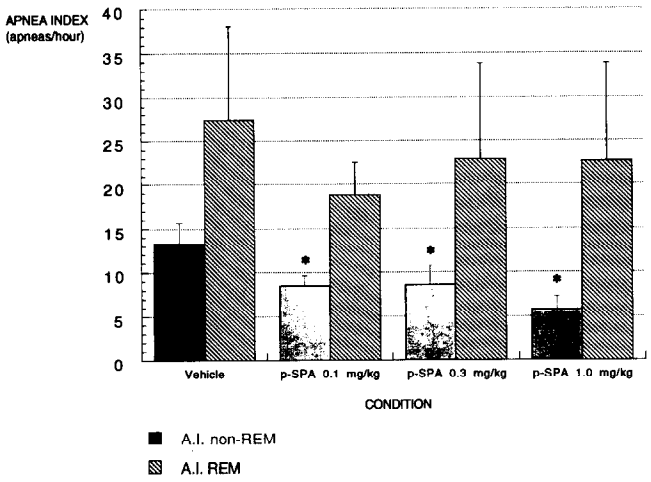

FIG. 4. Comparison of integrated postsigh and spontaneous AI during non-REM and REM conditions for vehicle and *p*-SPA at 0.1, 0.3, and 1.0 mg/kg. All doses of the drug significantly reduced AI exclusively during non-REM (*$p < 0.05$ related to vehicle).

Temporal Course of AI During Sleep

No trend was noted in sleep AI during the recording period. All three doses of *p*-SPA tended to maintain non-REM AI below baseline levels during the 6-h recording period (Fig. 5).

Effects of p-*SPA on Sleep*

As depicted in Fig. 6, *p*-SPA at doses of 0.1 and 0.3 mg/kg did not affect sleep efficiency in relation to baseline. A

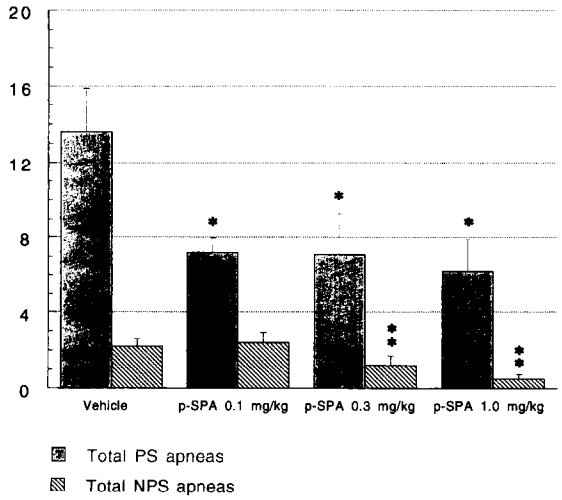

FIG. 3. Postsigh (PS) and spontaneous (NPS) AI after the administration of vehicle, 0.1, 0.3, or 1.0 mg/kg of *p*-SPA. All doses significantly reduced postsigh AI (*$p < 0.05$ related to vehicle), whereas spontaneous AI showed a decrease with 0.3 and 1.0 mg/kg doses (**$p < 0.05$).

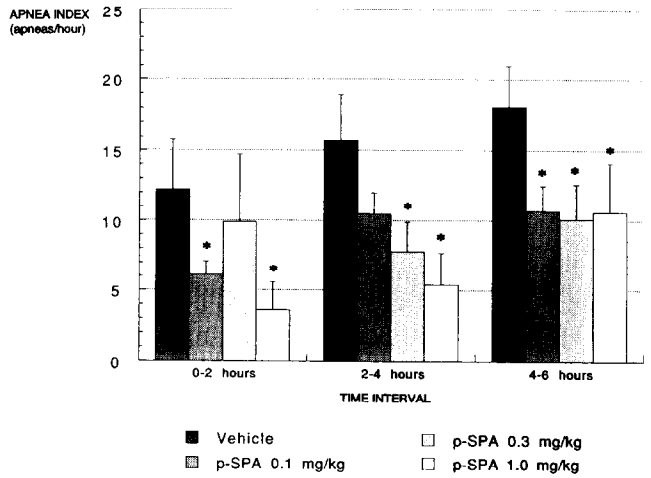

FIG. 5. Temporal variation of apnea index by 2-h intervals in all conditions. The bars marked with asterisk indicate significant reduction in AI compared to vehicle treatment within the same time interval (*$p < 0.05$). All doses showed a persistent effect during the 6-h recording.

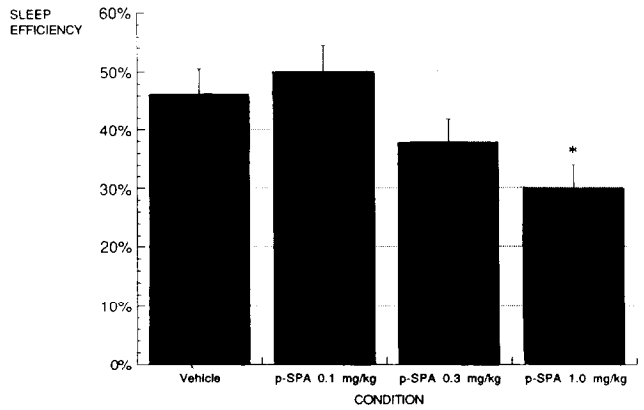

FIG. 6. Effects of *p*-SPA on sleep efficiency (non-REM + REM/360 × 100) during the 6-h recording period. Only 1.0 mg/kg of *p*-SPA reduced the amount of sleep in approximately 30% (*$p < 0.05$ related to vehicle).

significant reduction to approximately 60% of the baseline sleep efficiency was observed after the 1.0 mg/kg dose. This reduction resulted from a decrease in the amount of REM sleep ($p < 0.05$).

DISCUSSION

The present study supports our hypothesis that stimulation of peripheral adenosine A_1 receptors reduces the incidence of sleep apneas in the rat, an effect that occurred at all examined doses. Moreover, at doses of 0.1 and 0.3 mg/kg *p*-SPA, apnea suppressions occurred without changes in sleep efficiency or sleep architecture. At 0.1 mg/kg the suppression of apnea was restricted to postsigh apneas whereas 0.3 and 1.0 mg/kg *p*-SPA were also able to reduce spontaneous apneas. At all doses studied the apnea-suppressant effect was limited to non-REM sleep.

We have previously reported that systemic administration of either A_1 and A_2 adenosine receptor agonists (L-PIA and CGS 21680) can reduce the incidence of sleep apneas in the rat (11). This effect could be due to either (or both) peripheral or central effects because both compounds are known to enter the central nervous system. The similarities between the present effects following the administration of a selectively peripheral adenosine A_1 receptor agonist and our previously reported results further support our hypothesis that adenosinergic modulation of apnea expression may be mediated by stimulation of peripheral receptors in rats. The likelihood that adenosine suppresses apneas by stimulation of peripheral chemoreceptors is suggested by numerous reports (7–9,12).

The mechanisms underlying apneas in rats, as in man, are poorly understood. Thomas et al. (19) have demonstrated a differential response of postsigh and spontaneous apneas to several conditioning protocols, and have suggested that these two classes of apnea may be governed by different mechanisms. For this reason, we separately characterized the incidence of postsigh and spontaneous apneas. However, our results do not show clear differential responses of postsigh vs. spontaneous apneas to *p*-SPA to support the hypothesis that the two apnea types are generated, at least in part, by different mechanisms.

As we previously reported, the suppression of apnea associated with L-PIA and CGS 21680 occurred in parallel, with a dose-dependent decrease in sleep efficiency resulting primarily from a reduction in non-REM sleep duration. Conversely, at low and intermediate doses, *p*-SPA did not affect sleep architecture or efficiency, suggesting that control mechanisms of respiration and sleep may, to some extent, be independently manipulated. Nevertheless, sleep was suppressed by the highest dose of *p*-SPA. Prior evidence that *p*-SPA has no manifest central effects (6) indicates that its peripheral side effects (i.e., hypotension and hypothermia), which are known to suppress sleep, could well have induced changes in sleep architecture (3,4). Thus, the sleep-suppressant effect of *p*-SPA may be due to the appearance of side effects at high doses of the drug. These side effects could also be involved in the previously reported suppressant effects of CGS 21680 and L-PIA on sleep apnea (11), because both A_1 and A_2 compounds (agonists and antagonists) are well known to have cardiovascular impact (2,5).

Our present findings extend our previous data that adenosine A_1 analogues are able to reduce the incidence of sleep-related apneas in the rat. The existence of adenosine receptors in peripheral chemosensory organs, and the stimulatory action of adenosine analogues on them (7–9,12), is the most likely mechanism underlying their concomitant apnea-suppressant effect. Future studies using this novel peripheral adenosine analogue in rats with previously denervated chemoreceptors may be able to further support our hypothesis.

ACKNOWLEDGEMENTS

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