Pharmacology Biochemistry & Behavior, Vol. 22, pp. 889–891, 1985. 0091-3057/85 $3.00 + .00

BRIEF COMMUNICATION

p-Chloroamphetamine: Effect on Sleep and Respiration in the Rat

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Received 23 December 1982

DEMESQUITA, S. p-*Chloroamphetamine: Effect on sleep and respiration in the rat.* PHARMACOL BIOCHEM BEHAV 22(5) 889–891, 1985.—Rats, fitted with chronic EEG and EMG electrodes and a thoracic pneumograph, were monitored electrophysiologically for three successive days before and after an IP injection of *p*-chloroamphetamine (PCA) (2 mg/kg). During the 12 hours post PCA treatment, sleep onset was delayed, the percentage of Rapid Eye Movement (REM) sleep was decreased and the breathing rate during both the Non-REM (NREM) and REM sleep states was reduced. By 24 and 48 hours after the PCA injection, the sleep pattern and NREM respiratory rate had returned to control values; however, respiratory rate during REM sleep still tended to be decreased. The results suggest that PCA, at this dose, is capable of inducing insomnia and reducing REM sleep acutely without chronically altering the sleep pattern. The data also suggest that respiratory rate during sleep may decrease following PCA treatment.

*P*ara-chloroamphetamine Respiratory rate Rapid Eye Movement Sleep (REM) Brain serotonin

THE brain neurotransmitter serotonin has been postulated to be involved in the regulation of sleep [5], blood pressure, body temperature and respiration [1]. However the exact role of serotonin in the regulation of these basic functions is still unclear. This study used the serotonergic neurotoxin para-chloroamphetamine (PCA) as a pharmacological tool in an effort to determine the influence of brain serotonin on sleep architecture and respiratory rate during sleep. PCA has a biphasic effect on brain serotonin metabolism, first releasing and then depleting endogenous brain serotonin stores [10]. In this experiment sleep pattern and respiratory rate during NREM and REM sleep were examined immediately following PCA injection and again at 24 and 48 hours postinjection in an effort to identify acute and chronic changes in sleep and respiratory function related to PCA treatment.

METHOD

Animals

Six male Sprague-Dawley rats (274.2±7.6 g body weight) (mean±SE) were anesthetized (ketamine HCl 100.0 mg/kg; acepromazine malate 1.0 mg/kg) and implanted with chronic electrodes. Each rat was fitted with stainless steel screws placed bilaterally in the parietal bones and stainless steel wires hooked into the neck muscles in order to obtain a parietal electroencephalogram (EEG) and a nuchal electromyogram (EMG) respectively.

Apparatus

All rats were monitored while inside a sound attenuated, ventilated sleep chamber, maintained at 24±2°C with water and rat chow ad lib. Partially inflated balloons placed under the rat's cage and around the rat's chest were connected to pressure transducers in order to record whole body and respiratory chest movements respectively.

Procedure

Immediately following an injection of saline (2 mg/kg IP) each rat was monitored electrophysiologically for 3.5 hours. Two more control recordings were obtained (sans injection), one on each succeeding day. Four days after the last control recording each rat was given a single injection of PCA (2 mg/kg IP) and immediately monitored for 12 hours. Two more experimental recordings were obtained (sans injection), one on each succeeding day. With the exception of the recording session which lasted from 9:00 a.m. to 9:00 p.m. immediately following the injection of PCA, all other recording was done between 9:00 a.m. and 12:30 p.m.

Record Analysis

Each record was scored manually into Waking, NREM and REM sleep states according to the criteria below.

parameter	Wake	NREM	REM
body movement	±	−	−
EEG voltage	+	+++	+
EMG voltage	+++	++	+

A change in the state of consciousness was recognized if it persisted for at least thirty seconds. Sleep state parameters

TABLE 1
EFFECT OF PCA ON SLEEP PATTERN (N=6)

	Control*	0 hrs†	24 hrs‡	48 hrs§
NREM latency (min after recording start)	25.9 ± 4.3	372.7 ± 46.4¶	25.5 ± 5.9	32.2 ± 9.9
REM latency (min after NREM onset)	21.5 ± 4.1	165.5 ± 40.7††	26.0 ± 3.8	17.2 ± 2.6
TST % (total sleep time/ total recording time)	69.0 ± 5.9	31.7 ± 6.8**	61.9 ± 5.4	60.6 ± 5.7
REM % (total REM time/ total sleep time)	18.0 ± 1.7	4.7 ± 2.8**	19.3 ± 1.8	19.2 ± 1.7
Number of REMPS per hour of NREM time‡‡	5.1 ± 0.6	0.9 ± 0.4**	5.0 ± 0.8	6.6 ± 0.6
REMP duration (min)	2.6 ± 0.2	3.0 ± 0.7	3.0 ± 0.2	2.2 ± 0.2
NREM period duration prior to REM (min)	8.1 ± 0.9	7.2 ± 1.5	7.3 ± 0.7	5.8 ± 0.5††

*Mean ± SE of 3 control sleep recordings per rat (3.5 hours each).
†Mean ± SE of 12 hour recording immediately following PCA injection.
‡Mean ± SE of 3.5 hour recording 24 hours after PCA injection.
§Mean ± SE of 3.5 hour recording 48 hours after PCA injection.
¶Significantly different from control ($p<0.001$).
**Significantly different from control ($p<0.01$).
††Significantly different from control ($p<0.05$).
‡‡REMPS=REM periods.

were tabulated for each record (Table 1). Respiratory chest movements during the first minute of REM sleep and the preceding five minutes of NREM sleep were counted (Fig. 1). Each rat was used as its own control. Paired Student's *t*-test was used to test for differences in sleep pattern and respiratory rate in REM and NREM sleep before and after PCA treatment.

RESULTS

Sleep Pattern

PCA significantly ($p<0.001$) delayed the onset of NREM and REM sleep, decreased the percent of time spent asleep ($p<0.01$) and the proportion of sleep time spent in REM sleep ($p<0.01$) during the 12 hours following the PCA injection as compared to the control situation (Table 1). The dramatic reduction in REM percent was a result of the significant ($p<0.01$) decline in the number of REM periods (REMPS) occurring per hour of NREM sleep time and not by a decrease in REMP duration which was unchanged.

Respiration

Acutely sleep respiratory rate was significantly ($p<0.05$) decreased during the 12 hours following PCA treatment, with the REM sleep respiratory rate being significantly ($p<0.05$) more depressed than the NREM sleep breathing rate. At 24 and 48 hours following PCA treatment, the NREM respiratory rate was within normal limits while the breathing rate during REM sleep still tended to be slower than control, significantly ($p<0.05$) so at 24 hours post PCA treatment.

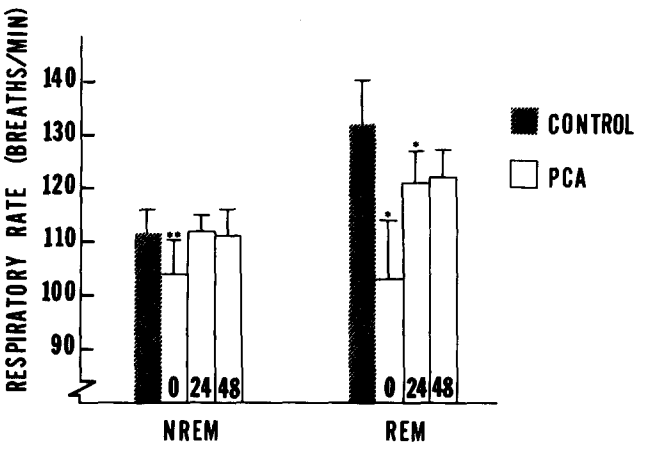

FIG. 1. Effect of PCA on respiratory rate during sleep (CONTROL—mean sleep respiratory rate from 3 control recordings, following saline injection (total of 12 NREM/REM epochs per rat); PCA—mean sleep respiratory rate at 0, 24 and 48 hours following para-chloroamphetamine injection based on 1.7, 4 and 4 NREM/REM epochs per rat respectively). *Significantly different from control $p<0.05$; **significantly different from control $p<0.01$.

DISCUSSION

A single dose of PCA produced insomnia lasting 6.5 hours followed by sleep with decreased REM percentage and reduced breathing frequency during both the NREM and REM

sleep states. *p*-Bromomethamphetamine and *p*-chloromethamphetamine, both halogenated amphetamine derivatives which cause brain sertonin depletion, have also been reported to produce insomnia acutely following a single injection [6]. However fenfluramine, a structural analog of amphetamine, which also causes long-term selective depletion of brain serotonin, has been reported to cause increased total sleep time in cats [4] while in rats a dose dependent suppression of both NREM and REM sleep has been reported [2]. Comparison of the various sleep studies using serotonin depleting drugs must be done cautiously since each drug has a separate temporal continuum of behavioral changes dependent on drug mechanism of action(s), dose and treatment schedule [2].

By 24 hours following PCA injection the sleep pattern in this study had returned to normal with no evidence of a REM sleep rebound. Similar results were found with *p*-bromomethamphetamine in rats [6] and low doses of fenfluramine in cats [4]. Since PCA neurotoxicity is dose dependent [3], the relatively low dose of PCA used in this study may account for the lack of chronic sleep pattern alteration. At 24 and 48 hours following injections of *p*-bromomethamphetamine and fenfluramine, brain serotonin levels have been found to be significantly depleted yet the sleep architecture is within normal limits. The results from this study further support the view [2] that rapid release of serotonin acutely causes suppression of sleep while the long-term depletion of brain serotonin stores does not prevent full recovery of sleep function.

The present study is the first to report a significant reduction in respiratory rate during sleep after PCA treatment. Rats with chronic central serotonergic deficiency have been reported to have increased sensitivity to the respiratory depressant effect of halothane [8]. Other studies using anesthetized [7] and awake [9] rats have reported stimulation of respiration following depletion of brain serotonin whereas respiratory stimulation has also been reported following intra-cerebroventricular injection of serotonin in anesthetized and conscious dogs [11]. These equivocal findings are probably due to differences in state of consciousness, drug dose and sampling times as well as species. Of interest in this study was the significant differential effect of PCA on REM respiratory rate as compared to NREM. Chronically, NREM respiratory rate returned to normal as did the sleep pattern, however the REM respiratory rate was still significantly slower 24 hours posttreatment. Since there is no direct correlation between sleep function and chronic whole brain serotonin depletion, it is possible that respiratory rate during REM sleep may be a more sensitive index of long-term brain serotonin depletion.

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