Effects of p-Chlorophenylalanine (p-CPA) on Sleep in Olfactory Bulb Lesioned Rats

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Abstract—The effect of p-CPA and 5-HTP followed by p-CPA on sleep was studied in rats with olfactory bulb lesions (O.B. lesioned rats). In these rats, electrodes were chronically implanted to record the EEG (frontal cortex and dorsal hippocampus), the cervical electromyogram and eye movements. The REM sleep stage was selectively decreased from 24 to 32 hours after 200 mg/kg of p-CPA in the sham lesioned rats, whereas both the slow wave sleep and REM sleep stages were markedly decreased by the same dose of p-CPA in the O.B. lesioned rats. In both sham and O.B. lesioned groups, the slow wave sleep and REM sleep stages decreased from 24 to 32 hours after 400 mg/kg of p-CPA and the percentage of decrease in the slow wave sleep stage was much larger with 400 mg/kg of p-CPA than with 200 mg/kg of p-CPA. However, the REM sleep stage disappeared from 24 to 32 hours after 200 mg/kg and 400 mg/kg of p-CPA. In the O.B. lesioned rats, the insomnia produced by 200 mg/kg and 400 mg/kg of p-CPA disappeared with 5-HTP (5 mg/kg). On the other hand, the insomnia produced by 200 mg/kg of p-CPA did not recur with 5-HTP in the sham lesioned rats, but with 400 mg/kg there was a recurrence. These results suggest that the enhanced effect of p-CPA and 5-HTP followed by p-CPA in the O.B. lesioned rats is due to changes in the sensitivity of the serotonergic system in the brain.

It has been reported that the administration of p-chlorophenylalanine (p-CPA), an inhibitor of 5-hydroxytryptamine (5-HT) synthesis, decreases the level of 5-HT in the brain (1) and produces an almost total insomnia in cats (2, 3), rats (4), monkeys (5) and rabbits (6). Olfactory bulb lesions in rats have produced changes in emotional response (7–9), general activity (10), visual discrimination (11), active and passive avoidance learning (12), and regulation of water balance (13) and taste preference and aversion (14).

Di Chiara et al. (15) observed that either bulbectomy or p-CPA alone induced muricide in only a certain percentage of animals, while the combination of the two was effective in 100% of cases. Further, they showed that muricide induced by bilaterally O.B. lesions was antagonized in animals in which brain serotonin had been increased by 5-HTP (5-hydroxytryptophan) or pargeline, a monoamine inhibitor.

When 5-HTP, the 5-HT precursor, is administered during insomnia induced by p-CPA, the REM sleep and slow wave sleep are restored in cats (2, 3). Our group has also reported that bilateral olfactory bulb lesions produced a decrease of REM sleep on the 7th, 21st and 28th day after operation (16).

1 Results of this investigation were presented at the 48th General Meeting of the Japanese Pharmacological Society, (April, 1976)
As there is apparently no information concerning the influence of p-CPA and 5-HTP followed by p-CPA on the sleep-wakefulness cycle in the O.B. lesioned rats, the present experiment was an attempt to explore the relationship between sleep and the 5-HT level by treating the O.B. lesioned rats with an inhibitor of synthesis and precursor of 5-HT.

**MATERIALS AND METHODS**

**Subjects:** Male Wistar rats weighing approx. 250 g were used at the beginning of the experiment. The animals were housed individually in plastic cages of 46 x 34 x 17 cm and food and water were provided *ad libitum*.

**Surgery:** Rats anesthetized with pentobarbital (45 mg/kg, i.p.) were placed on a stereotaxic instrument. After preparing the openings in the frontal cortex and dorsal hippocampus for electrode implantation, the olfactory bulbs were bilaterally exposed and removed by suction pump. An equal number of sham operated rats served as controls. All animals were sacrificed at the end of experiments and the lesions were verified histologically. It was confirmed that more than two thirds of the olfactory bulbs had been removed and some parts of olfactory nuclei had also been lesioned.

**Recording:** The treatments of p-CPA and 5-HTP followed by p-CPA were performed 28 days after O.B. lesions. A habituation time of 24 hr was allowed before start of the recording in a sound-proof room. The recording period was continued from 16:00 p.m. to 24:00 p.m..

**Drug treatment:** DL-parachlorophenylalanine (p-CPA) and D-5-hydroxytryptophan (5-HTP) solutions dissolved with 0.5% Tween were injected i.p. into the O.B. lesioned rats. The room temperature was kept at 25°C and humidity at 60%.

**RESULTS**

*Effect of p-CPA on sleep and waking levels in the O.B. lesioned rats*

According to change in spontaneous EEG, EOG and EMG, EEG levels in the O.B. lesioned rats were classified into the following 3 stages: wakefulness, slow wave sleep and REM sleep. Effects of p-CPA on the sleep-wakefulness levels were subsequently divided into 3 periods, according to the polygraphic results.

*Comparison between the sham lesioned and the O.B. lesioned rats before p-CPA:* In the O.B. lesioned rats, the REM sleep stage was significantly shortened 28 days after lesions com-

![Fig. 1. EEG pattern analysis as compared between sham and O.B. lesioned male Wistar rats.](image)
pared with the sham lesioned rats, without noticeable changes in the slow wave sleep and waking stages (Fig. 1).

*From the 24th to the 32nd hr following the injection of p-CPA:* With p-CPA (200 mg/kg, i.p.), the waking stage significantly increased and REM sleep stage markedly decreased without a noticeable change of the slow wave sleep in the sham lesioned rats. In the O.B. lesioned rats, both the slow wave sleep and REM sleep markedly decreased and conversely the waking stage markedly increased. The percentage of increase in the waking of the O.B. lesioned rats indicated a high value of 59.1% compared with the value of 41.2% in the sham lesioned rats (Fig. 2).

With p-CPA (400 mg/kg, i.p.), results similar to the injection of p-CPA (200 mg/kg, i.p.) were obtained. The percentage of increase in the waking of the O.B. lesioned rats was 68.5%, whereas the value in the sham lesioned rats was 54.1%. On the other hand, the percentage of decrease in the slow wave sleep and REM sleep of the O.B. lesioned rats was 19.4 ± 1.3% and 0.36 ± 0.2%, whereas these values in the sham lesioned rats were 33.12 ± 4.0% and 0.54 ± 0.3%, respectively (Fig. 3).

*From the 72nd to the 80th hr following the injection of p-CPA:* With p-CPA (200 mg/kg, i.p.), both the waking and slow wave sleep stages in the O.B. lesioned and the sham lesioned rats recovered to the values before the injection of p-CPA. On the other hand, the REM sleep showed a tendency toward increase, but the difference was not significant (Fig. 2).

With p-CPA (400 mg/kg, i.p.), results similar to the injection of p-CPA (200 mg/kg, i.p.) were obtained. However, in the O.B. lesioned rats, the percentage of REM sleep significantly increased compared with the value before p-CPA (Fig. 3.)
From the 168th to the 176th hr following the injection of p-CPA, both the waking and slow wave sleep stages showed normal values in two groups. In the sham lesioned rats given p-CPA, the REM sleep again significantly decreased (Figs. 2 and 3).

Effect of 5-HTP during insomnia produced by p-CPA in the sham lesioned and the O.B. lesioned rats

As shown in Fig. 4, when 5-HTP (5 mg/kg, i.p.) was administered 24 hr after p-CPA
Fig. 5. Effect of p-CPA (400 mg/kg) and 5-HTP (5 mg/kg) followed by p-CPA during the 8 hr recording session in sham and O.B. lesioned rats.

(200 mg/kg, i.p.), there was no significant effect of 5-HTP upon the states of sleep during the 8 hr of recording. However, the duration of the slow wave sleep stage was prolonged when compared with p-CPA alone. In contrast, in the O.B. lesioned rats, the administration of 5-HTP restored completely the slow wave sleep stage to the state before p-CPA.

In both the sham lesioned and the O.B. lesioned rats, as shown in Fig. 5, 5-HTP (5 mg/kg, i.p.) administered 24 hr after p-CPA (400 mg/kg, i.p.) restored the slow wave sleep stage to the state before p-CPA.

**Effect of p-CPA and 5-HTP followed by p-CPA on the total sleep in the sham lesioned and the O.B. lesioned rats**

As indicated in Table 1, in both the sham lesioned and the O.B. lesioned rats, the total sleep was significantly decreased by p-CPA (200 mg/kg and 400 mg/kg, i.p.). In particular, the percentage of decrease in the O.B. lesioned rats showed a higher value than that in the sham lesioned rats. When 5-HTP (5 mg/kg, i.p.) was injected at the time of insomnia 24 hr

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<tr>
<th>Table 1. Effect of p-CPA and 5-HTP followed by p-CPA on the total sleep in sham and O.B. lesioned rats</th>
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<tr>
<td><strong>Sham lesioned rats</strong></td>
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<tr>
<td><strong>Total sleep (%)</strong></td>
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<td>p-CPA (200 mg/kg)</td>
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<td>5-HTP (5 mg/kg)</td>
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<td>p-CPA (400 mg/kg)</td>
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* p < 0.01 when compared with control
after pretreatment with 200 mg/kg and 400 mg/kg of p-CPA in the O.B. lesioned rats, a complete disappearance of the insomnia occurred during the 8 hr of recording. In the sham lesioned rats, a slight insomnia induced by 200 mg/kg of p-CPA did not disappear with 5-HTP, however, when 400 mg/kg was given, the disappearance was to a great extent complete.

**DISCUSSION**

In our experiments with sham lesioned rats, the marked decrease in the total sleep was observed from the 24th to the 32nd hr after 400 mg/kg of p-CPA. The percentage of decrease was 31.6% and 93.7% in the slow wave sleep and REM sleep, respectively. It was reported by Florio et al. (17) that the three rats were administered 600 mg/kg of p-CPA given in two doses 24 hr apart and one animal was given 150 mg/kg for three consecutive days. No changes were noted in the animal treated with the lower dose but the animals given a higher dose showed a shift toward EEG desynchronization which reached maximum values between the third and fourth day from the last injection. Mouret et al. (4) reported that 500 mg/kg of p-CPA given i.p. is followed by a significant decrease in both slow wave sleep and REM sleep in rats. Some investigators observed that a high dose of p-CPA induced a decrease in slow wave sleep and REM sleep in the rat (4, 17, 18). Here our results are consistent with theirs. However, we found that the decrease in the total sleep did not continue for a long time and the sleep loss was considerably less than reported by Mouret et al. (4).

We found that the REM sleep stage in rats with bilaterally olfactory bulb lesions significantly decreased on the 7th, 21st and 28th days after surgery without noticeable changes in the slow wave sleep and the waking stages (16). Hence, in the present experiment, drugs were given 28 days after O.B. lesioning. It was induced that the pronounced decrease in the sleep period, particularly the slow wave sleep, occurred when p-CPA (200 mg/kg and 400 mg/kg, i.p.) was given to O.B. lesioned rats. Such insomnia induced by p-CPA completely disappeared with a small dose of 5-HTP (5 mg/kg, i.p.) only in the O.B. lesioned rats. Thus, the enhanced effect of p-CPA and 5-HTP followed by p-CPA was much larger in the O.B. lesioned rats than that in the sham lesioned rats.

There are reportedly no differences in brain 5-HT levels between the sham lesioned or unoperated controls and the bulbectomized rats (19, 20). We also have reported similar findings (21). Pohorecky and Chalmers stated (19) that in rats with unilateral olfactory bulb lesions, tyrosine and tryptophan levels in either the brainstem or the telencephalon remained unchanged. Neckers et al. (22) have recently reported that the bulbectomized mice had significantly less tryptophan hydroxylase in the brain, while neither 5-HTP decarboxylase nor tyrosine hydroxylase activity was affected. The decreased 5-HT level of rat brain treated with p-CPA is restored to only one third of the control level by giving i.p., 75 mg/kg of 5-HTP (1). Thus, the 5 mg/kg of the precursor given in the present work was apparently insufficient for recovery of 5-HT levels. Although the 5-HT level of the brain after 5-HTP was not determined in the O.B. lesioned rats, a possible mechanism by which insomnia induced by p-CPA is specifically sensitive to 5-HTP in the O.B. lesioned animals may be alteration of the serotonergic system other than restoration of the amine level. Possible
changes in the receptor activity and synthesis and catabolism of 5-HT produced in the O.B. lesioned rats treated with p-CPA are now being investigated.

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