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THE EFFECT OF 6-CHLOROTRYPTOPHAN ON SLEEP IN THE RAT

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Summary

The intraperitoneal injection of 6-CT, a highly specific inhibitor of tryptophan hydroxylase, induced a short lasting period of reduced PS and SWS. This sleep decrease was followed by a progressive rebound in both PS and SWS, which reached a maximum 12 hours after the injection. DL-5-HTP injection after 6-CT administration did not affect the initial sleep decrease observed, but the PS "rebound" was no longer observed. These results differ from those observed after inhibition of tryptophan hydroxylase by parachlorophenylalanine.

The possible role of some central serotonin (5-HT) containing neurons may be to trigger and to maintain the states of sleep. This is supported by a number of pharmacological and neurophysiological experiments :

Parachlorophenylalanine (PCPA) injections lead to a state of insomnia in both cats (1,12) and rats (15). The importance of this insomnia is correlated with the decrease in 5-HT levels due to inhibition of tryptophan-hydroxylase by the drug (2, 6, 10). The injection of 5-hydroxytryptophan (5-HTP) caused a bypass of the inhibited enzymatic step followed by a transitory return of sleep to its control levels (12).

However, 5-HTP is ineffective in reversing the state of insomnia induced by lesioning the cell bodies of the serotonergic neurons. After such a destruction, the intensity of insomnia is proportional to the number of neurons destroyed and the corresponding decrease in brain 5-HT levels (5, 8).

McGEER and PETERS have recently described a new class of tryptophan-hydroxylase inhibitors, the 6-halotryptophanes (4, 17). The inhibitory activity of these drugs reaches its maximum 2 hours

after the injection and fades out within 24 hours. This time course appears to follow the kinetics of reversible inhibition while the inhibition by PCPA appears to be irreversible and follows a progressive and long-lasting course (3). It was thus of interest to study the effects of one of these agents, 6-chlorotryptophan (6-CT), on the states of sleep in the rat.

Material and Methods

Male Wistar rats, weighing 200-250 gr, were chronically implanted with cortical and muscular electrodes under pentobarbital anesthesia. The cortical electrodes were stainless steel screws placed 2 mm lateral to the sagittal suture. The anterior electrodes were one millimeter rostral to the coronal suture, and the posterior ones were at the level of the lambdoid suture. Two flexible electrodes were inserted in the neck muscles in order to record their activity. The implantation was performed after at least one week of habituation to the light schedule of the laboratory (12 hours of light and 12 hours of dark). For one week following surgery, the rats were placed under recording conditions in single glass jars at a constant temperature (23°C). At the end of this adaptation period, at least three days of continuous (24 hours per day) control polygraphic records were taken. Each animal was given a daily injection of NaCl (pH : 9.5) for the three days preceeding the experiment and was used as his own control. The animals were subjected to one of the six following experiments :

1 - (12 rats) : injection of 200 mg/kg (0.836 mMol/kg) of 6-CT. This dosage was chosen because of its similarity to that used by McGEER (4). The 6-CT was first dissolved in a solution of 1N NaOH and then adjusted to a pH of 9.5.

2 - (22 rats) : injection of the same volume of NaCl at a pH of 9.5. (Control).

3 - (9 rats) : injection of 6-CT (200 mg/kg) followed 15 minutes later by an injection of DL-5-HTP (5 mg/kg).

4 - (8 rats) : control injection followed 15 minutes later by an injection of DL-5-HTP (5 mg/kg).

5 - (3 rats) : injection of 6-CT (200 mg/kg) followed after 15 minutes by an injection of L-tryptophan (200 mg/kg).

6 - (3 rats) : control injection followed 15 minutes later by an

injection of L-tryptophan (200 mg/kg).

All the injections were performed by the intraperitoneal (IP) route, at 10 a.m., which was 3 hours after the beginning of the light period and of the period when the rats would sleep the most (14).

The rats were continuously recorded throughout the experiment. The resulting records were scored to the nearest minute for their respective amounts of Waking (W), Slow Wave Sleep (SWS) and Paradoxical Sleep (PS). The criteria for the determination of these stages were those described by F. MICHEL et al (11).

Results

A - Effects of control injections

Following the first two control injections of NaCl, non-specific variations in W, SWS and PS were observed. After the third injection these records became stable. For a period of 30 minutes after each injection, the rats laid on the bottom of their cages, crawling instead of walking. At the end of the first hour after this injection, the rats' behavior appeared normal and PS and SWS amounts were within control values. Since unusual behavior in the first half hour followed all the injections, it appears to be due to the pH or salinity of the injection.

B - 6-CT 200 mg/kg

Though these rats follow a behavior pattern similar to that following control injections for the first hour, PS is completely absent for the first two hours after injection. After this period, PS (expressed on an hourly basis) progressively returns to control values and then overshoots and reaches a maximum 12 hours after injection valued at 175 % of the control value. (Figure 1, Table II). This increase in PS is due to an increased number of PS episodes whose duration remains unaltered (Figure 3). The same phenomenon, though to a lesser degree, was found for SWS. After an initial decrease (59 %) during the first two hours after injection there was a progressive increase with the maximum (132 %) 12 hours after injection (Figure 2, Table II). The PS and SWS levels returned to control levels 20 hours after the injection and no further changes were observed. Expressed on a 24 hour time base, from the time of injection, the amounts of PS and SWS are not significantly

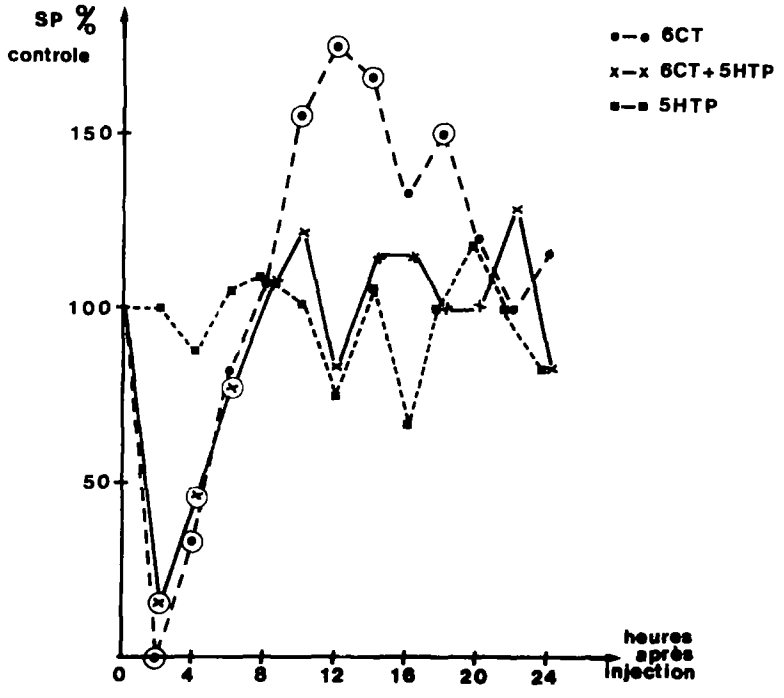

FIG. 1

Modifications of Paradoxical Sleep time after the different types of injections : PS is expressed on a 120 minutes base as percentage of control amounts.
6CT : injection of 6-chlorotryptophan (200 mg/kg) - 12 rats
5HTP : injection of DL 5 hydroxytryptophan (5 mg/kg) - 8 rats
6CT+5HTP : injection of 6CT plus injection of DL 5HTP 15 minutes later - 9 rats. The circled points represent significant differences ($p < .05$) as compared with control values.

TABLE II

Minutes of Paradoxical Sleep and Slow Wave Sleep per two-hour period following the injections.

Paradoxical sleep

Hours	10	12	14	16	18	20	22	24	2	4	6	8
C	6	18	17	13	9	4	6	6	6	5	7	6
6CT	0*	6*	14	14	14*	7*	10*	8	9*	6	7	7
C+DL 5HTP	6	16	18	14	10	3	7	4	6	6	7	5
6CT+DL 5HTP	1*	8*	13*	14	11	3	7	7	6	5	9	5

C :control injections (22 rats), 6CT :6chlorotryptophan(12 rats) and the association of the different types of injections with DL-5HTP (5-hydroxytryptophan): C+DL 5HTP (8 rats);6CT+DL 5HTP (9 rats). The data represent the average values obtained in each group. All the injections were performed at 10 a.m.

* Significantly different from the values after control injections ($p < .05$)

Slow Wave Sleep

Hours	10	12	14	16	18	20	22	24	2	4	6	8
C	47	72	65	60	43	25	41	36	44	36	49	47
6CT	27*	62*	65	61	50*	33	45	44*	49	41	49	42
C+DL 5HTP	47	73	64	60	44	22	44	35	44	40	54	45
6CT+DL 5HTP	29*	64*	72*	60	47	33	46	41	47	42	51	53

C :control injections (22 rats), 6CT :6chlorotryptophan (12 rats) and the association of the different types of injections with DL-5HTP (5-hydroxytryptophan): C+DL 5HTP (8 rats); 6CT+DL 5HTP (9 rats). The data represent the average values obtained in each group. All the injections were performed at 10 a.m.

* Significantly different from the values after control injections ($p < .05$)

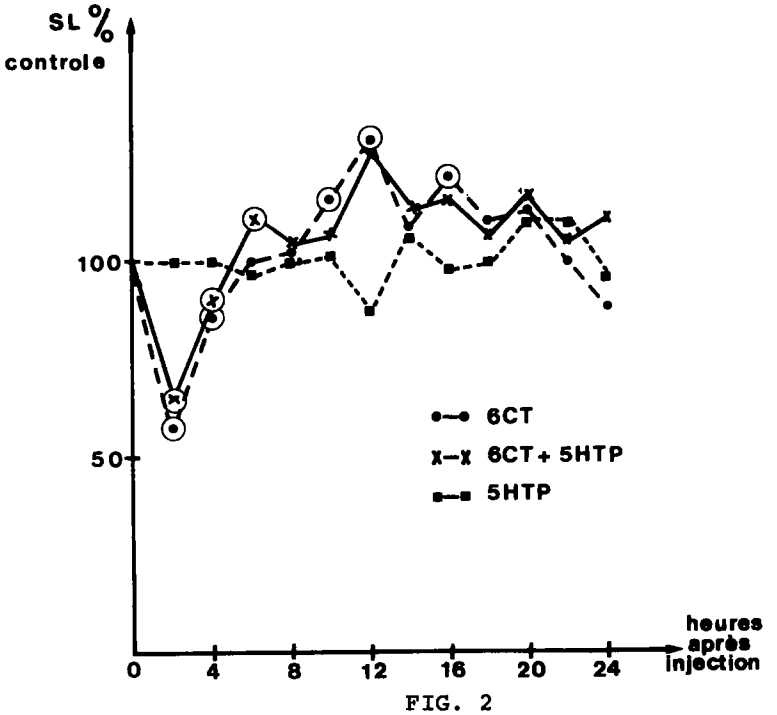

FIG. 2

Alterations of Slow Wave Sleep (SWS) after several types of injections. The results are expressed on a 120 minutes base as percentages of control amounts.
6CT :injection of 6-chlorotryptophan (200 mg/kg)-12 rats
5HTP :injection of DL 5 hydroxytryptophan (5 mg/kg)-8 rats
6CT + 5HTP :injection of 6CT plus injection of DL 5HTP 15 minutes later - 9 rats. The circled points represent significant differences ($p < .05$) as compared with control values.

different from the amounts after control injections. (Table I, Table II). The PS/SWS ratio parallels the changes in PS (Figure 4).

TABLE I

Paradoxical Sleep (PS) and Slow Wave Sleep (SWS) amounts, expressed in minutes, during the 24 hours after the injections.

	C	6-CT	6CT+DL 5HTP
Paradoxical Sleep	103±4	115±7	89±6 *
Slow Wave Sleep	566±14	572±35	578±38

C :control injections, 6CT :injections of 6-chlorotryptophan (200 mg/kg) (12 rats) 6CT+DL 5HTP :injections of 6CT followed by 5 mg/kg of DL 5-hydroxytryptophan (9 rats).
* Significantly different ($p < .05$ from the amounts after control injections.

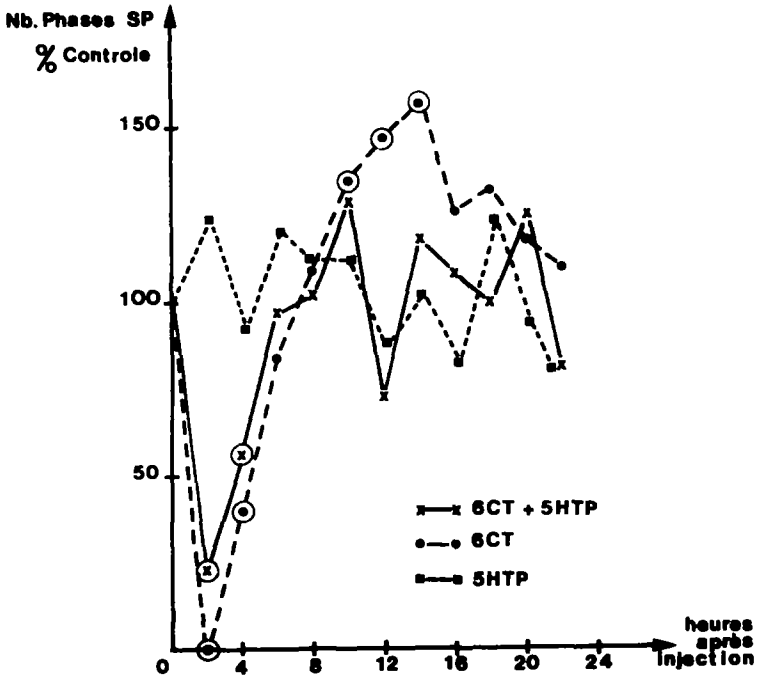

FIG. 3

Alterations of number of PS episodes after several types of injections. The results are expressed on a 120 minutes base as percentages of control amounts.
6CT :injection of 6-chlorotryptophan (200mg/kg)-12 rats
5HTP :injection of DL 5 hydroxytryptophan (5 mg/kg) -8 rats
6CT+5HTP :injection of 6CT plus injection of DL 5HTP 15 minutes later -9 rats. The circled points represent significant differences ($p < .05$) as compared with control values.

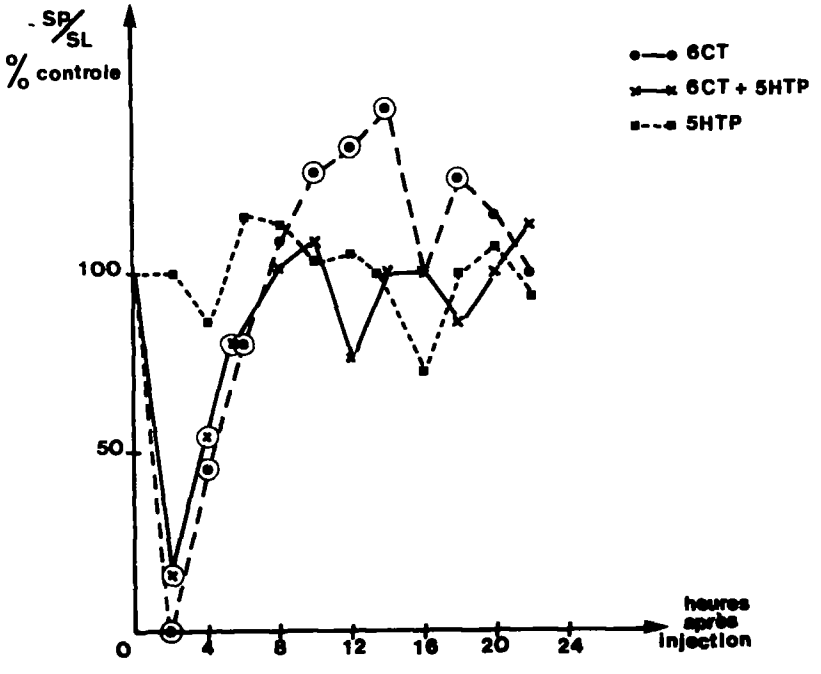

FIG. 4

Alterations in the ration PS/SWS after several types of injections. The results are expressed on a 120 minutes base as percentages of control amounts.
6CT :injection of 6-chlorotryptophan (200 mg/kg) -12 rats
5HTP :injection of DL 5 hydroxytryptophan (5 mg/kg) -8 rats
6CT+5HTP :injection of 6CT plus injection of DL 5HTP 15 minutes later -9 rats. The circled points represent significant differences ($p < .05$) as compared with control values.

C -6-CT followed by D-L 5-HTP

When injected just after a control injection, the doses used of D-L 5-HTP did not modify the effect of the control injection. However, the effects of 6-CT were altered by the injection of D-L 5-HTP. After the initial decrease in PS, which persisted, the rebound effect was no longer observed. Six hours after the injection, PS returned to control levels, but further increase did not occur (Figure 1). This absence of rebound was reflected in a significant decrease in PS over the 24 hour period after the injection (Table I, Table II). In contrast, the SWS variations observed after 6 CT alone, were unaltered by the D-L 5-HTP injection (Figure 2, Table II).

D - 6-CT followed by tryptophan injection

Tryptophan injections did not alter the effects of either control or 6-CT injections.

Discussion

These results demonstrate that the effects of 6-CT upon the sleep states of the rat are quite different from those of PCPA (15). The immediate and transitory decrease in PS and SWS after the 6-CT injection can be explained by the rapid and short-lasting action of the agent, whose effect reaches its maximum 2 hours after injection. However, the significant subsequent increase in PS and SWS seems difficult to explain. This increase in sleep has not been observed in rats after other pharmacological agents, such as PCPA (15) or nialamide (16), although these drugs cause an important decrease in both states of sleep. Moreover, this rebound effect after 6-CT makes up for the total sleep loss. This "ad integrum" recuperation or total replacement does not occur after PS deprivation induced by placing the rats in water tanks (9, 21). After this form of deprivation, the rebound makes up for only 60% of the sleep loss (9).

The rebound in PS after the 6-CT injection cannot be explained by the increase in SWS that was observed at the same time, because the PS/SWS ratio is significantly elevated. This increase in SWS also represents a puzzling problem, since this recuperation in SWS has previously never been described after pharmacological agents in the rat.

These results seem to conflict with the data for PCPA. However while these two compounds, 6-CT and PCPA, are thought to act upon the same enzyme, their mode of action appears to be quite different (4). After an injection of 6-CT (0.516 mMol/kg), tryptophan-hydroxylase activity as well as the brain level of 5-HT are back to control levels within 24 hours, while they are still decreased after an equivalent dose of PCPA (4). According to McGEER this difference might be explained by the existence of two pools of tryptophan hydroxylase in the brain, both being inhibited by PCPA and only one being affected by 6-halotryptophans (17). Recently, GAL (3) put forward the idea that PCPA may affect the primary structure of the enzyme by being incorporated at its active site.

This irreversible inactivation would then explain the long duration of this drug's action.

In view of the interrelationships between some noradrenergic neurons and PS (7, 18, 20), it could be that PCPA and 6-CT may act on these neurons. However, the action of PCPA on tyrosine hydroxylase is transitory and very weak, so the absence of any rebound from the PCPA insomnia, which lasts many days, could hardly be explained by the inhibition of this enzyme which lasts for only a few hours (15). On the other hand, the action of 6-CT seems to be very specific for tryptophan-hydroxylase.

One might also consider the hypothesis that 6-CT could simply bring about a shift in the circadian sleep rhythm. This could cause the predominant sleep phase to occur during darkness. Yet, such a phase shift occurs slowly with the use of pharmacological and physical agents, (13, 14). Moreover, it would stop abruptly at 6 a.m., and it is not likely that 6-CT could cause such abrupt reversals of the rhythm.

The effect of 5-HTP after 6-CT seems paradoxical and even more difficult to understand. An injection of 5-HTP in a cat under the influence of PCPA restores normal levels of sleep (11). Yet in the case of 6-CT, there are two main contradictions : first, 5-HTP has little or no effect on the decrease of SWS and PS levels nor on their return to control values. Second, 5-HTP appears to specifically block the PS rebound without affecting the course of the SWS rebound. Since there is not an inhibition of PS but a suppression of its rebound only, this effect could not be due to a non-specific action of 5-HTP on CA neurons (19).

Brain 5-HT turnover in these experiments has not yet been determined, but a correlation of the turnover of this amine with the insomnia and the sleep rebound may bring some explanation as to the action of 6-CT.

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