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Sleep–wake effects of *meta*-chlorophenyl piperazine and mianserin in the behaviorally depressed rat

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Abstract

The present study examined the effects of *meta*-chlorophenyl piperazine (mCPP) and mianserin on the sleep–wake cycle of the clomipramine-induced behaviorally screened depressed rats. Six-hour polygraphic recordings were made between 06:00 and 12:00 h, after a single injection of either saline or mianserin or mCPP into the lateral cerebral ventricle (i.c.v.) of both the depressed ($n = 12$) and control rats ($n = 12$). The injection of mCPP in the depressed rats caused a significant reduction in the total duration and number of rapid eye movement (REM) sleep episodes while it increased the REM sleep onset latency compared to the control saline injections. The injection of mianserin in the depressed rats also caused a significant reduction in the total duration and number of REM sleep episodes without changing the REM sleep latency. These results demonstrate for the first time that the central administration of mCPP and mianserin could act as an antidepressant in the clomipramine-induced rat model of depression.

Keywords: Animal model; Depression, endogenous; 5-HT (5-hydroxytryptamine, serotonin); REM (rapid eye movement) sleep; Mianserin; mCPP

1. Introduction

Endogenous depression is a major psychiatric disorder, characterized by a constellation of symptoms. The symptoms of this illness include depressed mood; loss of pleasure-seeking behavior like sex; changes in appetite; changes in sleep architecture; feeling of worthlessness or guilt; difficulty in thinking, concentrating or making decisions; and recurrent thoughts of death or suicidal ideation, plans or attempts (American Psychiatric Association, 1994). Sleep in endogenous depression is characterized by prolonged sleep latency, increased intermittent wakefulness, and early morning awakenings (Sharpley and Cowen, 1995). Depressed patients also exhibit reduction in the duration of stages 3 and 4 slow-wave sleep (SWS) with increased duration of stage 1 SWS (Reynolds and Kupfer, 1987). The changes in rapid eye movement (REM) sleep include the appearance of disinhibited REM sleep with decreased latency of REM sleep onset and increased frequency of REM sleep, especially early in the night (Reynolds and Kupfer, 1987). REM sleep in depression is also characterized by higher intensity phasic REM activity (Reynolds and Kupfer, 1987; Thase et al., 2001; Hans-Peter et al., 2001; McCracken et al., 1997).

Considerable progress has been made in understanding the phenomenology of endogenous depression; however, the biological basis of this psychiatric disorder remains poorly understood. Search for the biological basis of depression has been carried out for many decades, and alterations in brain serotonergic mechanisms have long been thought to play a major role in the pathophysiology of depression. There is now considerable evidence to support the hypothesis that human endogenous depression results partly from decreased central serotonergic activity (Coppen, 1968; Yates et al., 1990). This hypothesis is supported by the following evidence: (1) serotonin (5-HT) levels in the brain, plasma, and platelet are low in depressed patients (Pare et al., 1969; Shaw et al., 1967); (2) reserpine, a drug, which depletes both 5-HT and catecholamines in the brain, can produce depression-like symptoms in 15% of mentally normal hypertensive patients (Bunney and Davis, 1965); (3) inhibition of 5-HT synthesis with *p*-chlorophenylalanine

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(PCPA) produces relapse of depression in patients who had responded to antidepressants (Shopsin et al., 1975, 1976); (4) monoamine oxidase inhibitors (MAOI), which elevate the 5-HT concentration in the brain, also act as antidepressants (Blair et al., 1986); (5) reduction of dietary L-tryptophan, a precursor of 5-HT, can induce depressive symptoms under some circumstances (Young et al., 1985); (6) in patients, tryptophan depletion reverses the antidepressant response of 5-HT uptake inhibitors (Delgado et al., 1990).

The invention of the selective serotonin reuptake inhibitors (SSRIs) has substantially affected the treatment strategy of depression. Maximum antidepressant efficacy is obtained when tricyclic antidepressants were combined with SSRIs (Nelson, 1997; Pinder, 1997). Sertraline, an SSRI, acts as an antidepressant and also decreases the duration of REM sleep (Harkin et al., 1999). In the depressive patients, locomotor agitation, changes in REM sleep, sexual and cognitive dysfunctions are attributed to a low serotonin function (Kinney et al., 1997).

To understand the mechanisms involved in the pathogenesis of endogenous depression, a rat model has been developed (Vogel and Vogel, 1982). In this rat model, chronic treatment of clomipramine in neonatal rat pups for 14 days produces a myriad of behavioral deficiencies and REM sleep disturbances in adulthood, which collectively approximate the symptomatology of endogenous depression (Vogel et al., 1990). In addition to behavioral changes, one recent neurochemical study demonstrated that in the neonatally clomipramine-treated adult depressed (CLI) rats, the levels of 5-HT are significantly lower in several regions of the brain compared to adult control rats (Mavanji and Meti, 1999). As the serotonergic system is known to be involved in the regulation of the sleep–wake cycle (Jouvet, 1972; Datta, 1997; Boutrel et al., 1999; Monti and Monti, 1999), in the present study, we hypothesized that the alteration of REM sleep in CLI rats is due to the reduction of 5-HT in the brain. To test this hypothesis, in the present study, we have examined the effects of intracerebroventricular (i.c.v.) administration of serotonergic drugs on the sleep–wake cycle of CLI rats.

2. Materials and methods

2.1. Animal model

The rats (Wistar) used in this study were obtained from the breeding facility at the National Institute of Mental Health and Neurosciences (NIMHANS, Bangalore, India). All animals were treated in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals and institutional guidelines. Experimental details for the development of CLI rats are described in detail elsewhere (Vogel et al., 1990; Mavanji and Meti, 1999). Briefly, 4 days after the delivery, only male pups were allowed to grow with the mothers. From neonatal days 8–21, half of those male pups received subcutaneous injections of clomipramine (22.5 mg/kg body weight, in 0.125 ml normal saline) and the other half of those male pups received only control vehicle (0.125 ml of saline) injections. Each of these pups received control vehicle or clomipramine twice daily with an interval of 12 h for 14 days. After weaning, all the pups were housed individually until 3 months old.

2.2. Subjects

Experiments were performed on 15 clomipramine-treated (CLI) and 12 control vehicle-treated adult male (control) rats of 3 months old, which were derived from separate litters. The rats were housed individually at 25 °C with food and water provided ad libitum. Lights were on from 06:00 to 18:00 h (light cycle) and off from 18:00 to 06:00 h (dark cycle). At the time of behavioral testing and polygraphic recording, the body weight of the rats was between 250 and 300 g.

2.3. Behavioral testing

When these rats attained the age of 3 months, they were tested for the extent of depression by studying the alterations in shock-induced aggressive behavior as described in detail elsewhere (Vogel et al., 1988; Mavanji and Meti, 1999). Rats were paired randomly for behavioral testing. Each control or clomipramine-treated pair of rats was subjected to behavioral testing for 4 consecutive days. On day 1, the rat pair was left in the testing chamber for 10 min to undergo habituation. After habituation, rats were housed in pairs throughout the end of day 4 testing. On test days 2–4, after 2 min of habituation, 100 electrical shocks (each shock: 4 mA with a duration of 0.4 s) were delivered to the grid floor of the behavioral chamber with a fixed intershock interval of 7.5 s. The neonatal clomipramine-treated rats that showed more defensive behaviors like defensive upright posture, submissive crouching, and lying in supine position in more than 70% of the trials were considered as depressed (Vogel et al., 1988; Mavanji and Meti, 1999).

2.4. Drugs and vehicle for injections

The drugs included 1-(*m*-chlorophenyl) piperazine (mCPP; Sigma, St. Louis, MO), a 5-HT receptor agonist, and mianserin (Aldrich, Milwaukee, WI), an antidepressant (Fuller et al., 1981; Pinder and Fink, 1982). The drug mCPP binds to the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5HT_{1D}, and 5-HT_{2C} receptors (Lawlor et al., 1991). In rodents, it binds more potently to the 5-HT_{1B} receptors (Lawlor et al., 1991). This drug also binds to the 5-HT transporter and increases the levels of extracellular 5-HT (Victor et al., 1982). The drug mCPP is commonly used as a pharmacological probe to assess central serotonergic function in humans and laboratory animals (Fuller et al., 1981; Sanford et al., 1994). The

drug mianserin is used as an antidepressant in humans (Mendlewicz et al., 1985). Mianserin acts as an antagonist at 5-HT_{1A} and 5-HT_{2C} receptors (Gleason et al., 2001). The metabolite of mianserin, demethylmianserin, inhibits reuptake of 5-HT at the nerve terminals (Victor et al., 1982). These drugs were dissolved in 0.9% saline, and solutions were adjusted to pH 7.0. This 0.9% saline was also used for the control vehicle microinjection.

2.5. Surgical preparation

As described in detail recently (Datta et al., 2000, 2001), behaviorally screened 12 CLI and 12 control rats were anesthetized with pentobarbital (40 mg/kg, i.p.) and implanted with electrodes for scoring sleep and wakefulness. During the same surgical procedure, a stainless steel guide tube (21-gauge) was aimed for the lateral ventricle (1 mm posterior, 1.5 mm lateral, and 3.5 mm dorsal from the bregmax) with the use of the stereotaxic coordinates of Paxinos and Watson (Paxinos and Watson, 1986). Following a postsurgical recovery period of 5–7 days, the rats were trained to sleep in the sleep-recording chamber for 7 days between 06:00 and 12:00 h (first half of the sleep period in rats). Since the sleep parameters of endogenously depressed patients are significantly different than normal subjects during the first half of a typical sleep period, in this study, all adaptation and experimental recording sessions were performed during the first half of the sleep period in rats (between 06:00 and 12:00 h).

2.6. Intracerebroventricular injections and the experimental design

After the baseline recording sessions, injection sessions began. Six-hour (between 06:00 and 12:00 h) injection recording sessions were begun after a single injection of 1 μl control saline or mCPP (4 μg in 1 μl) or mianserin (4 μg in 1 μl) into the lateral ventricle, as described in our earlier publications (Datta et al., 1992, 2001). In individual rats, each injection was separated by at least 3 days in a random order. Each animal received two injections (saline and mCPP or mianserin) in two different recording sessions.

2.7. Determination of wakefulness and sleep stages and data analysis

For determining the possible effects on sleep and wakefulness, three behavioral states were distinguished based on the visual scoring of polygraphic records (simultaneous recordings of electroencephalogram, electromyogram, and electrooculogram) as described earlier (Datta and Hobson, 2000). The behavioral states of wakefulness, SWS and REM sleep were scored in successive 30 epochs. The polygraphic measures provided the following dependent variables that are quantified for each recording session: (1) total time spent in wakefulness, SWS, and REM sleep in 6-h recording session; (2) latency to the first episode of REM sleep from the start of SWS; and (3) mean number of REM sleep episodes in 6-h recording session. The duration of the slow-wave sleep preceding and continuous with the first REM sleep episode was taken as the REM sleep latency. Analysis of variance (two-way ANOVA) and post hoc *t*-tests (least significant difference, LSD) were used to examine the effects of control saline vs. mCPP or mianserin on dependent variables of control and CLI rats.

3. Results

3.1. Effects of neonatal clomipramine treatment on aggressive behavior of adults

Based on the behavioral criteria, described in Materials and methods, 12 of the 15 CLI rats were considered as depressed. The aggressive scores of the remaining three clomipramine-treated nondepressed rats were not included in the analysis of data. Aggression scores of these 12 CLI rats were compared with aggression scores of 12 control rats. The mean aggression scores of these 12 clomipramine-treated rats (10.6 ± 5.1; mean ± S.D.) were significantly less ($P < 0.001$) than the mean aggression scores in the 12 control rats (63.6 ± 10.7). These 12 CLI and 12 control rats were used to study the effects of mCPP and mianserin on the sleep–wake cycle.

3.2. Effects of clomipramine-induced depression on wakefulness and sleep stages

In the CLI animals, during the 6-h recording sessions, the time spent in wakefulness (215.9 ± 25.6 min; mean ± S.D.) and SWS (138.1 ± 20.4 min) was not significantly different from the time spent in wakefulness (203.2 ± 30.2 min) and SWS (153.3 ± 18.6 min) by the control animals. However, in the CLI animals, the time spent in REM sleep (15.5 ± 3.1 min) was significantly ($P < 0.001$) higher compared to the time spent by the control rats (6.8 ± 1.9 min). Having documented the increase in the duration of REM sleep in the CLI rats, we looked at the changes in latency and number of REM sleep episodes in these rats. The REM sleep latency in the CLI rats (11 ± 1.2 min) was significantly ($P < 0.001$) shorter compared to the REM sleep latency in the control rats (17.3 ± 2.2 min). However, the mean number of REM episodes during the 6-h recording periods was significantly ($P < 0.001$) higher in the CLI rats (11.6 ± 2.1) compared to the number of REM sleep episodes in the control rat (5.6 ± 1.2).

3.3. Effects of mCPP on wakefulness and sleep stages

In the 6-h recording sessions, in the control rats, the total time spent in wakefulness and SWS after injection of mCPP was not significantly different from the total time spent in

Table 1
Effects of mCPP and mianserin on the total time (mean ± S.D. in min) spent in wakefulness and slow-wave sleep

Group + treatment	Wakefulness	Slow-wave sleep
Control + saline	211.43 ± 49.3	163.6 ± 33
Control + mCPP	179.6 ± 23.7	166.8 ± 23.2
Control + mianserin	175.1 ± 10.6	172.3 ± 34.9
CLI + saline	220.4 ± 40.6	144.8 ± 40.4
CLI + mCPP	194.1 ± 21.4	165.8 ± 30.8
CLI + mianserin	124.3 ± 36.7	212.1 ± 48.2

In the control and neonatally clomipramine-treated (CLI) adult rats, the total time spent in wakefulness and slow-wave sleep after i.c.v. application of mCPP and mianserin were not significantly different than after i.c.v. application of control saline.

wakefulness and SWS after injection of control vehicle (Table 1). Similarly, the latency, number of episodes, and total time spent in the REM sleep were not significantly different after mCPP injection compared to that after saline injection in the control rats (Fig. 1).

The injection of mCPP in the CLI rats caused a significant reduction ($P<0.05$) in the total time spent in REM sleep compared to the injection of saline (Fig. 1). After mCPP injection, the total time spent in REM sleep in the CLI rats was reduced to the level of total time spent in REM sleep by the control rats. Similarly, the mCPP injection in the CLI rats caused significant ($P<0.001$) reduction in the number of REM sleep episodes and increased REM sleep latency compared to the microinjection of saline (Fig. 1). When the total time spent in REM sleep, REM sleep latency, and total number of REM sleep episodes were compared between after injection of mCPP in the CLI rat and after saline injection in the control rats, they were not significantly different (Fig. 1). Like in the control rats, in the CLI rats, the total time spent in wakefulness and SWS was not significantly different after mCPP injection compared to

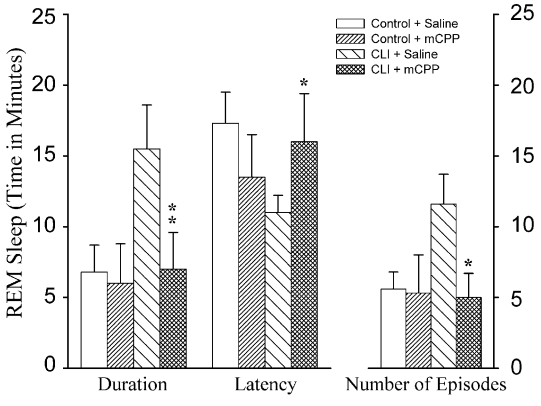

Fig. 1. Effects of mCPP on the duration (mean ± S.D.), latency, and number of episodes of REM sleep in the prenatally clomipramine-treated adult (CLI) and control rats. Note that, in the CLI rats, mCPP injection (i.c.v.) caused a reduction in the duration as well as the number of episodes of REM sleep and increased the REM onset latency compared to the saline injection. *$P<0.05$ and **$P<0.01$.

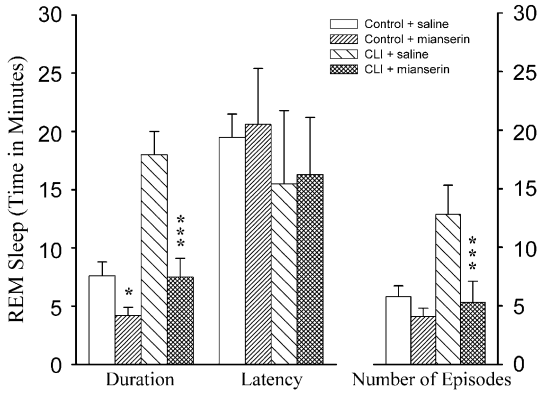

Fig. 2. Effects of mianserin on the duration (mean ± S.D.), latency, and number of episodes of REM sleep in the prenatally clomipramine-treated (CLI) and control adult rats. Note that, in the control rats, mianserin injection (i.c.v.) reduced the duration of REM sleep compared to saline injection. In the CLI rats, mianserin injection caused a reduction in the duration and number of episodes of REM sleep compared to saline injection. *$P<0.05$ and ***$P<0.001$.

after saline injection (Table 1). These results indicated that the injection of mCPP is capable of restoring REM sleep disturbances in the CLI rats.

3.4. Effects of mianserin on the wake–sleep stages

In the control rats, compared to after control injection, injection of mianserin did not change the total time spent in wakefulness and SWS significantly (Table 1). Injections of mianserin in the control rats significantly decreased the total time spent in REM sleep ($P<0.001$) compared to the saline injections (Fig. 2). However, in the same control rats, the REM sleep latency and the number of REM sleep episodes after mianserin remained comparable to after saline injection.

In the CLI rats, the total time spent in wakefulness and SWS after mianserin injection was not significantly different from the time spent in wakefulness and SWS after saline injection (Table 1). To our surprise, similar to the mCPP effect, mianserin injection in the CLI rats also significantly reduced the total time spent in REM sleep ($P<0.001$) and the total number of REM sleep episodes ($P<0.001$) compared to after saline injection (Fig. 2). The REM sleep latency in the CLI rats was not significantly different after mianserin and saline injection.

4. Discussion

The principal findings of this study are: (1) REM sleep onset latency is significantly shorter in the clomipramine-treated rats than saline-treated rats; (2) the total number of REM sleep episodes is significantly higher in the clomipramine-treated rats than in the saline-treated rats; (3) the total amount of REM sleep in the clomipramine-treated rats is

significantly more than the saline-treated rats; (4) mCPP treatment in the clomipramine-treated depressed rats decreases total amount of REM sleep by increasing REM sleep onset latency and by decreasing total number of REM sleep episodes; (5) mianserin treatment in the clomipramine-treated depressed rats decreases total amount of REM sleep by decreasing total number of REM sleep episodes. These results show for the first time that the REM sleep disturbances in the clomipramine treatment-induced depressed rats could be attenuated by increasing serotonergic activity of the brain.

4.1. Methodological consideration

The following discussion is organized around the premise that our results are relevant to the pathophysiological mechanisms for REM sleep disturbances in endogenous depression. In order to study sleep architecture in a nocturnal animal, it is important to conduct recording sessions during the day, when rats spend most of the time asleep (Datta et al., 2000, 2001). In this study, we have recorded the sleep–wake cycle in the first half of the day because the sleep disturbances in the depressed patients are prominent during the first half of the night (Reynolds and Kupfer, 1987). To understand the serotonergic mechanisms involved in the pathogenesis of endogenous depression, we chose to study neonatal clomipramine treatment-induced rat model of depression because this animal model produces a myriad of behavioral deficiencies in adulthood, which, collectively, approximate the symptomatology indicative of clinical endogenous depression (Vogel et al., 1990). For example, clomipramine-treated animals exhibit decreased sexual activity (Neil et al., 1990; Vogel et al., 1990), increased open-field locomotor activity (Hartley et al., 1990), and decreased aggressive behavior (Vogel et al., 1988). In adulthood, the neonatal clomipramine-treated rats exhibit REM sleep abnormalities that are similar to those seen in human endogenously depressed patients (Vogel et al., 1990). It has also been shown that treatment with imipramine together with REM sleep deprivation normalizes the deficits in sexual activity and aggression in the clomipramine treatment-induced depressed rat (Vogel et al., 1990). This finding is consistent with the finding in humans that REM sleep deprivation alleviates the symptoms of depression (Vogel et al., 1975). A study by Vogel et al. (1988) showed diminished aggression in the clomipramine-treated depressed rats. In the present study, 80% of the clomipramine-treated rats showed diminished aggressive behavior and, hence, were considered as depressed. Although the diminished aggressive behavior is commonly used for the identification of depressed rat, however, this behavior is not a primary diagnostic criterion for the endogenous depression in human. The increased REM sleep in the CLI rats at the age of 3 months in this study is in accordance with an earlier study that showed increased REM sleep in the neonatal clomipramine treatment-induced depressed adult rat (Vogel et al., 1990).

To understand the role of the serotonergic system, in the present study, we have used the serotonergic drugs, mCPP and mianserin. The mCPP was used because this drug has been used frequently as a pharmacological probe to investigate the brain serotonergic system in the rat (Fuller et al., 1981; Sanford et al., 1994). The mCPP is known to increase serotonin release (Bauman et al., 1993) and reduce REM sleep in humans (Lawlor et al., 1991). In addition to the mCPP, in this study, we have used a tetracyclic compound, mianserin, because it has antidepressant efficacy (Pinder and Fink, 1982) and has an inhibitory action on REM sleep in human depressives (Mendlewicz et al., 1985).

4.2. REM sleep in CLI rat

Increased total time spent in REM sleep and shorter REM sleep latency in the clomipramine treatment-induced depressed adult rats may be due to the reduction in brain level of serotonin. Indeed, in our earlier study, we have shown that these clomipramine treatment-induced depressed adult rats' brain level of serotonin is significantly less than the control rats (Mavanji and Meti, 1999). Also, it has been shown that neuronal activity of dorsal raphe serotonergic neurons is decreased in clomipramine-induced depressed rats (Kinney et al., 1997). A number of studies have shown that the level of 5-HT in the dorsal raphe is at a minimum during REM sleep (Portas and McCarley, 1994; Portas et al., 1998). Systemic and central administration of serotonin and its agonists suppress REM sleep (Datta et al., 1987; Quattrochi et al., 1992; Stickgold et al., 1993; Sanford et al., 1995; Horner et al., 1997). The results of this study together with the evidence above support our hypothesis that the REM sleep disturbance in the clomipramine treatment-induced depressed adult rats is due to the reduction in brain level of serotonin.

4.3. REM sleep effects of mCPP REM sleep in CLI rat

The present study demonstrated that i.c.v. injection of mCPP reduces the total duration of REM sleep in CLI rats. The reduction in the total amount of REM sleep is a result of the reduction in total number of REM sleep episodes and increased REM sleep latency. Since mCPP treatment increases serotonin release in the brain (Bauman et al., 1993) and administration of serotonergic agonist reduces REM sleep (Quattrochi et al., 1992; Stickgold et al., 1993), the reduction in REM sleep after mCPP treatment in the CLI rats may be due to increased serotonin release in the brain. Our interpretation is consistent with the earlier suggestion that serotonin exerts an inhibitory influence on REM sleep and depression (McCarley, 1982). Systemic administration of mCPP reduces the total amount of REM sleep in the normal rat (Dugovic and Van Den Broeck, 1991) and in humans (Yoshiaki et al., 1993). However, in our study,

mCPP application into the cerebral ventricle of the normal rat did not change the total amount of REM sleep. This difference in the effect of mCPP in the normal rat may be due to the difference in route of administration. Since mCPP inhibits acetylecholine release (Vizi et al., 1981), it is also possible that mCPP reduced REM sleep in depressed rats by reducing acetylecholine release in the brainstem REM sleep generator (Gillin et al., 1982, 1993; Sitaram et al., 1982; Datta, 1995).

4.4. REM sleep effects of mianserin in CLI rat

The present study showed that after i.c.v. injection of mianserin, the CLI rats spent significantly less time in REM sleep. This effect of mianserin is consistent with earlier studies that showed mianserin decreased the duration of REM sleep in both depressive patients and normal subjects (Mendlewicz et al., 1985; Tormey et al., 1980). There are two possible mechanisms by which mianserin administration could increase serotonin availability and reduce REM sleep. First, mianserin could bind to the 5-HT autoreceptors. Mianserin has a high affinity for 5-HT_1 receptors (Richard, 1987) and these receptors are closely related to the release modulating autoreceptors. Mianserin binding to those 5-HT autoreceptors would enhance further release of serotonin in the brain. Second, demethylmianserin, a metabolite of mianserin, could increase brain level of serotonin by acting as a serotonin reuptake inhibitor (Victor et al., 1982). It is also possible that the reduction of REM sleep after mianserin treatment may be due to the inhibition of alpha-2 noradrenergic receptors (Python et al., 1997; Victor et al., 1982).

4.5. Possible significance

The present study is significant because it provides some of the first experimental evidence showing that the REM sleep disturbances in the CLI rat could be normalized by central administration of mCPP and mianserin. Since the REM sleep effects of mCPP and mianserin in CLI rats and depressed humans are very similar, it is reasonable to suggest that the clomipramine-induced depression in the rat is an excellent model to study the pathophysiological mechanisms of human endogenous depression. Finally, this study highlights the critical importance of the normal prenatal and neonatal development of the serotonergic system for its later functions (Datta et al., 2000).

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