Effects of selective serotonin reuptake inhibition on vigilance and quantitative EEG

Ph.D. theses

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1 Introduction

Nowadays, depression is the leading cause of incapability. Symptoms such as loss of interest, feelings of low self-worth, poor concentration, changes in body weight, fatigue and death-related thoughts almost always accompanied by sleep disturbances. Indeed, primary sleep disturbances are important risk factors for depression as it was investigated.

Investigations aiming the neurotransmitter systems underlying of depression related sleep disturbances bring us closer to the proper treatment of depression. Although several generations of antidepressants were used in case of depression, almost half of the treated patients do not respond to treatments. This is the main reason to study the therapeutically successful antidepressants in animal studies and to collect more detailed results regarding their mechanism of action. That is highly recommended in case of sleep studies, because sleep architecture of rodents is an adequate model of human sleep. Thus, our investigations can facilitate the development of new agents for satisfactory treatment of non-responder depressed patients. About 90% of depressed patients complaint about sleep disturbances that are characteristics of depression: explicit increase of REM sleep quantity, decrease of REM sleep latency, decrease of deep slow wave sleep quantity, increase of early morning awakenings and increase of sleep latency. Most of the antidepressants decrease the time spent in REM sleep of experimental animals, normal controls and depressed patients.

Mood-improving and antidepressant-like effects of sleep deprivation using various methods regarding duration and timing of deprivation has been proved in both animal and human studies. Sleep deprivation method called *flower pot* is an effective method to execute a highly selective REM sleep deprivation in order to analyze changes during the rebound sleep period.

We used escitalopram, the most selective and highly effective selective serotonin reuptake inhibitor (SSRI) with favorable side-effect profile, and analyzed its effects on vigilance stages using rats in physiological conditions or after long term sleep deprivation.

2 Aims

Acute effects of escitalopram (2 mg/kg or 10 mg/kg) on vigilance stages were analyzed using electroencephalography (EEG) on freely moving male rats (Vas et al. 2013). Changes in sleep architecture during sleep rebound caused by the long term sleep deprivation (72 h) that was followed by acute escitalopram treatment were also analyzed using the more effective dose of escitalopram (Kátai et al. 2013).

The aims of our experiments were to answer the following questions:

- How does 2 mg/kg dose of escitalopram administered immediately at the beginning of the passive phase affect sleep-wake parameters of freely moving rats with normal sleep-wake cycle?
- How does 10 mg/kg dose of escitalopram administered immediately at the beginning of the passive phase affect sleep-wake parameters of freely moving rats with normal sleep-wake cycle?
- How does 2 mg/kg dose of escitalopram administered immediately at the beginning of the passive phase affect quantitative EEG of freely moving rats with normal sleep-wake cycle?
- How does 10 mg/kg dose of escitalopram administered immediately at the beginning of the passive phase affect quantitative EEG of freely moving rats with normal sleep-wake cycle?
- How does 10 mg/kg dose of escitalopram administered after 72-hour-long sleep deprivation change occurrence of vigilance stages of rats during the 3-hour-long rebound sleep period?

3 Materials and methods

All experiments were carried out using male Wistar rats. Rats were kept under controlled environmental conditions (temperature at 21±1°C and a 12-h light-dark cycle starting at 10:00a.m.), and food and water were available ad libitum during the whole experiment.

3.1 Sleep studies

Rats participating in experiment investigating sleep effects of escitalopram received 1 ml/kg solution of the following treatments, intraperitoneally; right after the lights on and before the EEG recording was started:

- Veh group (n=9): treated with vehicle (control)
- SSRI-2 group (n=13): treated with 2.0 mg/kg escitalopram (SSRI)
- SSRI-10 group (n=12): treated with 10.0 mg/kg escitalopram (SSRI)

REM sleep deprivation (RD) was carried out using methods of *flower pot*. During this experiment each RD rats was placed on a round platform (diameter = 6.5 cm, surface was 0.5 cm above the water level) situated in the middle of a round water tank (diameter = 41 cm). The only place where rats were able to sleep was the platform. However, they were able to stand on it, but were not able to lie down or curl up due to its dimension. Consequently, rats were not able to reach REM sleep, because during REM sleep one of their limbs would reach the water due to the muscle atony. Besides, slow wave sleep could be measured during sleep deprivation, although in a bit decreased quantity. The REM sleep deprivation was started at lights on and finished 72 h later. During this period the control rats were kept in their home cages (HC) in the same room as the REM sleep deprived animals. Both HC and RD groups were weighed, treated and reattached to EEG recoding cables right after the 72 hours at lights on. EEG was recorded 3-h-long during the sleep rebound period.

Rats participating in experiment investigating sleep effects of escitalopram after 72-h-long sleep deprivation, received 1 ml/kg solution of the following treatments, intraperitoneally:

- HC-Veh group (n=5): kept in home cage (HC) under undisturbed circumstances, received vehiculum (Veh) treatment,
- HC-SSRI group (n=5): kept in home cage (HC) under undisturbed circumstances, received 10.0 mg/kg escitalopram (SSRI) treatment,
- RD-Veh (n=6): 72-h-long REM sleep deprived (RD), received vehiculum (Veh) treatment,
- RD-SSRI (n=5): 72-h-long REM sleep deprived (RD), received 10.0 mg/kg escitalopram (SSRI) treatment

In addition to the EEG signals led from the surface of the brain, activity of the neck muscle (electromyogram, EMG) and motility of the animals were also recorded. The sleep analysis program gave the opportunity to use the quantitative EEG analysis, based on the Fast Fourier Transformation. Namely, this method supplies information about the frequency related power density (μV^2) of the EEG signals.

The following vigilance stages and calculated parameters were analyzed based on the EEG:

- Wakefulness
    - Active wakefulness
    - Passive wakefulness
- Slow Wave Sleep
    - Light Slow Wave Sleep
    - Deep Slow Wave Sleep
        - Latency of Deep Slow Wave Sleep
- Intermediate Stage
- REM sleep
        - Latency of REM sleep
        - First REM sleep item
        - Average number of REM sleep items
        - Average duration of REM sleep items

3.2 Statistical analysis

Sleep parameters were evaluated by repeated measures two-way ANOVA. Tukey honest significant difference (Tukey HSD) test was used for post hoc analysis. Level of significance was $p<0.05$.

4 Results

Effects of the selective serotonin reuptake inhibitor escitalopram treatment on vigilance and quantitative EEG of freely moving rats and on rebound sleep after 72-hour-long sleep deprivation can be summarized as follows:

- Escitalopram effectively decreased – already in 2 mg/kg dose – the time spent in REM sleep, the average number of REM sleep items and increased the latency of REM sleep.
- Regarding the above mentioned parameters, the 10 mg/kg dose of escitalopram caused the same changes but in a more robust way during a prolonged time.
- Escitalopram in 2 mg/kg dose increased the time spent in intermediate stage but 10 mg/kg dose decreased it.
- Main effect of escitalopram using quantitative EEG was the decrease of power density at 8 Hz during both active wakefulness and REM sleep.
- Both SSRI treatment and sleep deprivation had effect on REM-sleep rebound.
- Despite the high REM sleep pressure caused by the REM sleep deprivation procedure, escitalopram had the ability to suppress REM sleep rebound.
- Above mentioned effects of escitalopram treatment were accompanied by the decrease of the average number of REM sleep items right after the administration of the treatment.
- Additionally to the REM sleep decreasing effect of the SSRI treatment it increased the time spent in deep slow wave sleep during the REM sleep rebound after sleep deprivation.

5 Summary

Nowadays, depression is the leading cause of the incapability. Besides the psychic symptoms of depression about 90% of depressed patient complaints about sleep disturbances.

Investigations aiming the neurotransmitter systems underlying of depression related sleep disturbances bring us closer to the proper treatment of the depression. This is the main reason to study the therapeutically successful antidepressants in animal studies and to collect more detailed results regarding the mechanism of their actions. It is highly recommended in case of sleep studies, because sleep architecture of rodents is an adequate model of human sleep. Increased REM sleep pressure should be emphasized among of the sleep disturbances accompanied depression, namely decrease of REM sleep latency and increased time spent in REM sleep.

We used escitalopram, the most selective, highly effective and well tolerated selective serotonin reuptake inhibitor (SSRI), and analyzed its effects on vigilance stages using electroencephalography (EEG) in rats. Acute effects of two doses (2 mg/kg and 10 mg/kg, i.p.) of escitalopram on vigilance stages and quantitative EEG were studied in our basic experiment. Furthermore, effects of 10 mg/kg acute escitalopram treatment on vigilance stages occurred during sleep rebound period following the 72-h-long sleep deprivation were studied using the *flower pot* method.

Escitalopram treatment effectively decreased – already in 2 mg/kg dose – the time spent in REM sleep, the average number of REM sleep items and increased the latency of REM sleep in our experiments. The 10 mg/kg dose of escitalopram caused the same changes but in a more robust way during a prolonged time. Main effect of escitalopram using quantitative EEG was the decrease of the power density at 8 Hz during both active wakefulness and REM sleep. Despite the high REM sleep pressure caused by REM sleep deprivation procedure, escitalopram has the ability to suppress REM sleep rebound accompanied by the increase of quantity of the deep slow wave sleep.

Based on our EEG results, the acute escitalopram treatment has prominent effect on sleep architecture not only in normal condition but after sleep deprivation. In this way, our study investigating effects profile of escitalopram contributes to the information gathering about neuronal background of SSRI treatments.

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