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Short communication

Orexins cause epileptic activity

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ABSTRACT

Orexins have been implicated in the regulation of sleep–wake cycle, energy homeostasis, drinking behavior, analgesia, attention, learning and memory but their effects on epileptic activity are controversial. We investigated whether intracortical injections of orexin A (100 pmol) and B (100 pmol) cause epileptic activity in rats. We observed epileptic seizure findings on these two groups rats. Orexin A and B also significantly increased total EEG power spectrum. Our findings indicate that orexins cause epileptic activity.

1. Introduction

The orexins (OXs), orexin A and B, also known as hypocretins (hypocretin 1 and 2), are neuropeptides derived from the same precursor molecule, prepro-orexin, synthesized in the lateral hypothalamic area [5,31]. Orexinergic neurons project widely to numerous brain regions including cerebral cortex, thalamus, hypothalamus, nucleus accumbens, brain stem and spinal cord [4,27,29]. OX receptors (OX1 and OX2) are expressed in these areas especially in the cortical regions, hippocampus, thalamic, hypothalamic and brain stem nuclei [22,40]. It has been reported that OXs may play a role in various physiological functions including the energy homeostasis [12,32,44], sleep–wake cycle [32], drinking behavior [19], analgesia [25], attention [10], learning [36] and memory [1,15]. Although it was shown in numerous studies that orexins have neuroexcitatory effect [5,42], there were few studies, which investigate orexin–epilepsy relationship [8,18,30]. In a previous study, it was shown that after generalized convulsions, the levels of orexin A decrease in cerebrospinal fluid [30]. In another study it has been reported that orexin A decreased bicuculline-induced epileptic activity according to *in vitro* experiments [8]. On the other hand, in our previous study, we showed that orexins enhance the cortical epileptic activity induced by intracortical application of penicillin-G [18]. The findings of previous studies investigating orexin–epilepsy relationship are controversial [8,18,30] and orexin–epilepsy relationship is not clear yet. Based on our previous findings we thought that orexins induce epileptic activity without using any epileptogenic agent. Therefore the aim of this study was to investigate whether orexins cause epileptic activity in rats.

2. Materials and methods

2.1. Animals and study design

All of the experiments were approved by the Committee of Animal Care at Pamukkale University and the experiments were performed according to the guidelines (NIH, UCSF) on animal use. Twenty adult male Wistar Albino rats weighing 243 ± 26 g (mean ± SD) were used. All of the rats were maintained in a 12-h light/dark cycle environment (lights on 7:00–19:00 h) at a temperature of 23 ± 2 °C and 50% humidity. Rats had access to food and water *ad libitum*.

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Table 1
EEG power spectrum values of experimental groups.

Group	Before	30 min	60 min	90 min	120 min
OXA	$8.31 \times 10^{-11} \pm 1.88 \times 10^{-11}$	$1.32 \times 10^{-09} \pm 2.45 \times 10^{-10}$	$1.94 \times 10^{-09} \pm 3.51 \times 10^{-10}$	$3.65 \times 10^{-09} \pm 6.18 \times 10^{-10}$[a]	$3.89 \times 10^{-09} \pm 5.07 \times 10^{-10}$[a]
OXB	$5.30 \times 10^{-11} \pm 1.18 \times 10^{-11}$	$9.05 \times 10^{-10} \pm 3.08 \times 10^{-10}$	$1.37 \times 10^{-09} \pm 4.13 \times 10^{-10}$	$2.19 \times 10^{-09} \pm 4.61 \times 10^{-10}$[b]	$2.53 \times 10^{-09} \pm 4.08 \times 10^{-10}$[a]
Saline	$6.52 \times 10^{-11} \pm 1.42 \times 10^{-11}$	$8.23 \times 10^{-11} \pm 2.37 \times 10^{-11}$	$1.05 \times 10^{-10} \pm 3.17 \times 10^{-11}$	$1.26 \times 10^{-10} \pm 2.63 \times 10^{-11}$	$8.44 \times 10^{-11} \pm 1.41 \times 10^{-11}$
Control	$9.90 \times 10^{-11} \pm 2.85 \times 10^{-11}$	$7.67 \times 10^{-11} \pm 1.97 \times 10^{-11}$	$1.20 \times 10^{-10} \pm 3.51 \times 10^{-11}$	$5.18 \times 10^{-11} \pm 1.87 \times 10^{-11}$	$4.24 \times 10^{-11} \pm 1.09 \times 10^{-11}$

Values are presented as mean ± S.D. OXA, orexin A; OXB, orexin B.
[a] $p < 0.01$, different from before orexin injection.
[b] $p < 0.05$, different from before orexin injection.
$n = 5$ for each group.

The rats were randomly assigned to the following four groups ($n = 5$ for each group). Intracortical (i.c.) orexin A (OXA, 100 pmol, dissolved in 2 μl saline), orexin B (OXB, 100 pmol, dissolved in 2 μl saline) and saline (2 μl) was administered into the rats in the groups 1, 2 and 3, respectively. Group 4 received no drug or saline.

2.2. Anesthesia and experimental procedure

The rats were anesthetized with ketamine/xylazine (90 and 10 mg/kg respectively i.p.) and their heads were shaved. Then, the rats were placed on a stereotaxic instrument (Stoelting Co., USA) and their head's were disinfected with batticon (Batticon, Adeka Co., Turkey) and incised from mid-frontal to mid-occipital. After the bregma was exposed, a hole was drilled by a dental drill to a point that was determined to be the rat brain atlas of Paxinos and Watson [28] (from bregma: 0.7 mm anterior, 2.0 mm right laterally, 2.0 mm vertically). 100 pmol orexin A and 100 pmol orexin B were dissolved in 2 μl saline and were administered to the primary motor cortex by microinjector (Hamilton Co., USA) to group 1 and group 2, respectively. Similarly, 2 μl saline injection was administered to the same area in group 3. Aliquots of orexins were prepared and frozen at −20 °C for each experiment and thawed and dissolved in 2 μl saline immediately before use. Orexin A and B were purchased from Sigma–Aldrich Co., Germany.

2.3. EEG record and analyses

Two AgCl flat electrodes were placed on the scalp for bipolar EEG recording; one of them was placed on the right parietal area, and the other on the mid-occipital area. A ground electrode was placed on the tail of the rat. EEG was recorded by PowerLab 8/SP data acquisition system and Chart 5.2.2 program (ADInstruments Co., Australia). The recording parameters were as follows: 0.3–100 Hz low and high frequency filter, 50 Hz notch filter, and a recording speed of 25 mm/s. Whenever additional anesthesia was needed, it was administered to the rats. The rats were observed and recorded during the experimental period. Thirty second artifact-free epochs were chosen from the EEG recordings as the samples of cortical activity, at before the orexin/saline injection and 30th, 60th, 90th and 120th min of orexin/saline injection. The power spectrum analysis of these EEG samples was performed by Chart 5.2.2 software program (ADInstruments Co., Australia). Spectral power values were transferred to the SPSS 10.0 program and analyzed by repeated measures ANOVA and *Post hoc* Tukey test. *P* value of <0.05 being considered as significant.

3. Results

We observed epileptic seizure findings on the group 1 and group 2 rats, which were applied 100 pmol (i.c.) OXA and 100 pmol (i.c.) OXB, respectively. After 25–32 min OXs administrations, tonic–clonic contractions on the left anterior extremities of the rats were observed. The contractions spreaded to the left posterior, right anterior and posterior extremities, tail and whole body of the rats. The severity of the contractions increased and continued to the ending of the experiments (Until 120 min after OXs administrations). In the group 1 rats (OXA applied) the contractions were observed more severe than group 2 rats (OXB applied). However there were no epileptic seizure findings in the saline and control groups. Also, total EEG power spectrum was increased significantly in the orexin A and orexin B groups, at 90 and 120 min after the orexin injections compared to the values of before orexin application (Table 1). Whereas, total EEG power spectrum did not significantly change in the control and saline groups (Table 1).

4. Discussion

In this study, it was shown that orexin applications caused apparent increase on total EEG power spectrum. In addition to EEG findings, during experiments epileptic activity findings including tonic–clonic contractions on the whole extremities, tail and body of the rats were observed by physical observations. Also, similarly to previous studies [6] the effect of OXA was more potent than that of OXB in this study. On the other hand in the saline and control groups neither contractions nor change in EEG findings were observed.

In the previous studies using 100 pmol orexin A or B epileptic contractions or epileptic seizure findings in EEG were not reported [6,7,24,37,38,43]. There may be numerous reasons for this situation. One of them can be the localization of the brain area where orexins were injected. In the present study, orexins were injected to the primary motor area. On the other hand in the previous studies orexins were injected at the same dose to different brain areas including the basal forebrain, nucleus basalis, substantia innominata, magnocellular preoptic nucleus, nucleus accumbens, lateral cerebral ventricle and rostral lateral hypothalamic area [6,7,24,37,38,43]. Several factors (e.g. orexin receptors density) in these areas may change the effects of orexins. The second reason may be the origin of the orexins which were provided from different companies having possibly different efficiency. In this study, orexins were bought from Sigma–Aldrich. In the previous studies orexins were bought from different companies (American Peptides, Sunnyvale, CA, USA and Peptide Institute, Minoh, Japan) [7,24,37,38,43]. Thorpe and Kotz reported that orexins which have been bought from different companies had no similar effects on appetite stimulation [38]. Therefore orexins, which were provided from different companies, may have different effects on epileptic activity. The third reason may be, in the previous studies, the same doses of orexins might have induced focal epileptic activity but it could not be observed. In our study, because of orexins' injection to the primary motor area we could observe physically apparent epileptic activity. Also, in the determination of focal epileptic activity, appropriate EEG recording is important. However in some of the previous studies using 100 pmol orexin, EEG was not recorded [24,37,38,43]. On the other hand in the two of the studies using 100 pmol orexin, EEG was recorded and changes on arousal pattern of EEG caused by orexins were reported. But in these studies, epileptic activity findings in EEG were not reported [6,7].

According to some other previous studies, orexins have neuroexcitatory effect and may cause behavioral convulsion activity [5,13,42]. Our results confirm these reports.

It is known that during epileptic activity, balance between glutamate and GABA release is abolished [2,34]. In the previous studies it was reported that orexins increased glutamate [16,17,41] and GABA [23,41] release. On the other hand there are several studies reported that orexins decreased glutamate [11] and GABA [39] release. In another study, it has been shown that NMDA receptors are also related with epileptic activity [21]. In rats, the results of previous studies for explanation of the mechanisms of epileptic activity caused by orexins are straightforward.

Another possible cause of orexin-induced epileptic activity may be the direct effect of orexins on neuronal depolarization. According to *in vitro* experimental studies, orexins cause depolarization in neurons [33] and increase in firing frequencies of neurons [3,9]. Orexins may form direct excitatory effects on neurons via increasing the influx of sodium [20], activate sodium–calcium exchanger pump [9], increase influx of calcium [26,42] or decrease efflux of potassium [14]. The importance of intracellular calcium increase in terms of neuronal excitability and epileptic activity is known [35]. For this reason, this mechanism also mediates the effects of orexins on the central nervous system. Hence these effects of orexins, which facilitate neuronal depolarization, support epileptic activity and may explain the increase in total EEG power spectrum in our study.

In conclusion, in this study, we showed that intracortical applications of orexins caused epileptic activity, but the mechanisms of this effect are not clear.

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Conflict of interest

The authors declare that there is no conflict of interest.

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