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Central Action of a Fixed Valerian-Hops Extract Combination (Ze 91019) in Freely Moving Rats

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Abstract
Due to the electrochemical nature of the communication structure of the brain an intimate relationship between neurotransmitter activity on one side and field potentials (EEG) on the other side have been reported. From this it can be assumed that the electrical activity reflects the net effect of drug action. The influence of increasing doses of Ze 91019 on field potentials in chronically instrumented, freely moving rats was registered for 4 hours in order to test its action on the central nervous system. Doses of 200 mg and 250 mg valerian siccum extract (with additional 48 or 60 mg hop siccum extract) increased the spectral power in the delta, theta and alpha frequency bands of the frontal cortex suggestive of attenuated cholinergic transmission. Since adenosine administration into the basal forebrain also increases the low frequency activity in the frontal cortex it can be assumed that adenosine in the basal forebrain mediates these frequency changes by attenuating the frontal cholinergic system. Thus, Valerian and the fixed valerian hop extract combination Ze 91019 seems to cause this change of spectral power acting as an adenosine A1 receptor agonist. The results are therefore in line with the view that the fixed extract combination Ze 91019 works as an adenosine agonist via inhibition of cholinergic transmission leading to sedation and sleep.

Key words: Valerian extract, Hop extract, CNS, Sedative, Sleep, Adenosine receptors, Agonist, Cholinergic system, EEG, Animal study

Introduction

Sleep architecture and sleep propensity are determined by a two-process model. The model postulates that a homeostatic process rises during waking and declines during sleep. The time course of the homeostatic process was derived from electroencephalogram slow wave activity (spectral power in the 0.75-4.5 Hz band) [1]. Cluster analysis of the mean power maps of the non rapid-eye-movement (nonREM) sleep electroencephalogram revealed a topographic segregation into distinct frequency bands with a frontal predominance in the delta and alpha band. Prolonged wakefulness by sleep deprivation induced an increase in power in the low frequency range (1-10.75 Hz) predominantly in the frontal region, which may be indicative for increased sleep pressure [2].

Extracellular adenosine level selectively increased in basal forebrain during prolonged wakefulness by sleep deprivation and promotes the transition from wakefulness to slow wave sleep by inhibiting cholinergic and non-cholinergic wakefulness-promoting basal forebrain neurons at the adenosine A1 receptors [3]. Delivery of adenosine into the basal forebrain increased sleepiness and decreased wakefulness [4]. Dialysis delivery of an A1 receptor agonist to the pontine reticular formation similarly decreased acetylcholine release [5]. Administration of adenosine kinase inhibitors, by which the extracellular adenosine levels becomes elevated, augmented EEG slow waves indicating modulation of the slow wave activity via adenosine A1 receptors [6]. Today adenosine is accepted to work as an endogenous somnogen, i.e. an agent that promotes sleep [7]. Adenosine promotes the induction of sleep by two complementary cellular mechanisms, first by the direct inhibition of the wakefulness-promoting neurons in the cholinergic zone of the basal forebrain, and secondly by indirect activation of sleep promoting neurons in the preoptic/anterior hypothalamic area [3].

Valeriana officinalis has been used since ancient time as a sleep aid. The entire extract must be seen as the active principle even if a few compounds have been selected, which also demonstrate effects [8]. More recently valerian extract was shown to bind to adenosine A1 receptors and acts at the cellular level as a partial agonist [9]. The binding capacity of the pure valerian extract was still present in a fixed valerian hop extract combination (Ze 91019). An agonistic action of valerian at the adenosine A1 receptors was confirmed in another experimental setting using brain slices. This valerian action was dose dependent [10]. The fixed valerian hop extract combination (Ze 91019) diminished time and dose related the central action of caffeine, an adenosine A1 receptor antagonist, in volunteers, indicating bioavailability after oral administration as well as competing activity at the target, i.e. the central nervous system [11].

The aim of the present experiments in chronically instrumented rats is to demonstrate that the orally administered extract combination Ze 91019 elevates the power in slow wave activities in the frontal cortex. Such elevation might be interpreted as wakeful inhibi-

tion or somnogenic action of the extract combination mediated at the adenosine A1 receptors in the basal forebrain.

Material and Methods

Recording of field potentials from the depth of the brain provides a sensitive assay to detect changes in brain electrical dynamics, which are related to behaviour and to central neurotransmission. The methodology has already been described in details [12]. Briefly, adult Fisher rats (5-8 month of age and day - night converted) were stereotactically implanted with 4 bipolar concentric steel electrodes. The electrodes were placed 3 mm lateral within the left hemisphere. Anterior coordinates are 12.2, 5.7, 9.7 and 3.7 mm for frontal cortex, hippocampus, striatum and reticular formation (according to the atlas of Paxinos and Watson, 1982). A base plate carrying 4 bipolar stainless steel semi-micro electrodes (neurological electrodes "SNF 100" from Rhodes Medical Instruments, Inc., Summerland, CA 93067, USA) and a 5-pin-plug was fixed to the skull. The distant recording spot of the electrode was the active electrode whereas the proximal spots of the four electrodes were connected to each other to give a common reference. The base plate was carrying a plug to receive later on the transmitter (weight: 5.2 g including battery, 26x12x6 mm of size).

Animals were given two weeks for recovery. After recovering the transmitter was plugged in for adaptation and control experiments with saline were performed. During the recording rats could move freely. The principles of laboratory animal care were followed in all trials and the local authorities responsible for animal care allowed the performance according to German Health Guidelines.

EEG signals were recorded from frontal cortex, hippocampus, striatum and reticular formation. Signals were wirelessly transmitted by a radio-telemetric system (Rhema Labortechnik, Hofheim, Germany, using 40 Megahertz as carrier frequency) and were amplified and processed to give power spectra of 0.25 Hz resolution. In short, after automatic artefact rejection signals were collected in sweeps of 4 s duration and Fast Fourier transformed using a Hanning window. Sampling frequency was 512 Hz. Four values were averaged to give a final sampling frequency of 128 Hz, well above the Nyquist frequency. The resulting electrical power spectra were divided into 6 specially defined frequency ranges (delta: 0.8 - 4.5 Hz; theta: 4.75 - 6.75 Hz; alpha1: 7.00 - 9.50 Hz; alpha2: 9.75 - 12.50 Hz; beta1: 12.75 - 18.50 Hz; beta2: 18.75 - 35.00 Hz). Spectra were averaged in steps of 3 minutes each and displayed on-line. In an off-line procedure spectra were averaged to give 60 minutes or longer periods for further analysis and data presentation. After a pre-drug period of 45 minutes for baseline recording, drug effects were observed for 240 minutes thereafter. Changes of the recorded electrical power (μV2/sec) are given in percentage of the pre-drug values representing the average of n = 8 animals being exposed to one dosage of Ze 91019 per week. During the experiments the animals were observed with the aid of a video system in the dark under infrared illumination.

The reactions to Solutol (vehicle) and the different dosages were analysed with a total of 8 animals, which went into experiments repeatedly in groups of four with a drug-free interval of 1 week between successive trials. The data presented here are averages over 7-8 single experiments. Tablets containing the fixed extract combination Ze 91019 were pulverized by means of a mortar and suspended in 20 % Solutol (BASF, Mannheim, Germany). Dosages (mg/kg) refer to the amount of valerian siccum extract in the fixed extract combination, which, in addition, contains 60 mg hop siccum extract pro 250 mg valerian siccum extract.

To test for significant differences between drug action and the vehicle effect, the Mann-Whitney U-test was used and p-values less than 0.05 accepted to indicate significance.

Results

Application of dosages of 200 and 250 mg/kg resulted in an increase of delta and alpha2 power in the cortex and hippocampus starting with the second hour after injection (Fig. 1). These changes became more intense during the third and fourth hour. Changes became statistically significant for the 200 mg/kg dosage during the last hour. In general, strongest effects of Ze 91019 were detected in the frontal cortex followed by the hippocampus.

After a lag phase of 1 hour the pattern of changes remained very consistent over time. Due to the stable effects starting with the second hour data were averaged over the remaining time period. In the frontal cortex delta, theta and alpha2 frequency changes for the averaged time period are dose dependent (Fig. 2 and 3).

Discussion

Oral administration of the fixed valerian hop extract combination Ze 91019 remarkably induced spectral power increase in the low frequency activity, e.g. delta, theta and alpha frequency bands particular in the frontal cortex. This result confirms the hypothesis that the extract combination interacts with the adenosine A1 receptors in the basal forebrain and reduces wakefulness or increase sleep pressure in the frontal cortex, a basic structure for vigilance and alertness. This action may provide a scientific explanation for the efficacy of these plant extracts as sleep aid.

Similar results were obtained by local administration of adenosine or its agonist and, in addition, adenosine antagonists provoke the opposite effects [3-5, 11, 14]. By means of the quantitative EEG for a dose of 1200 mg of valerian extract an increase of power in the delta and theta band in volunteers have already been reported [15]. Furthermore, administration of the fixed extract combination in patients suffering from non-organic sleep disturbances revealed reduction in sleep latency and increase of slow wave sleep [16]. In sleep disturbed rats, administration of 1000 or 3000 mg valerian extract increased delta activity during non-REM

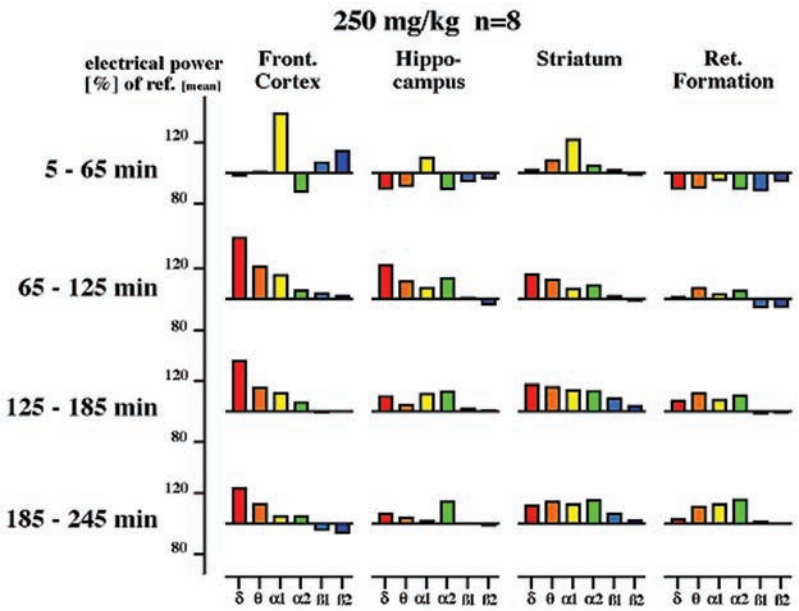

Fig. 1. Time course of frequency band changes after administration of 250 mg/kg valerian siccum extract (part of the extract combination Ze 91019). Single bars are representing delta (red), theta (orange), alpha1 (yellow), alpha2 (green), beta1 (blue) and beta2 (dark blue) frequencies. Values are given in % of 45 min base line recordings (=100%). Increases are shown upward, decreases downward. Values start 5 minutes after oral administration. Averages are given from 8 animals.

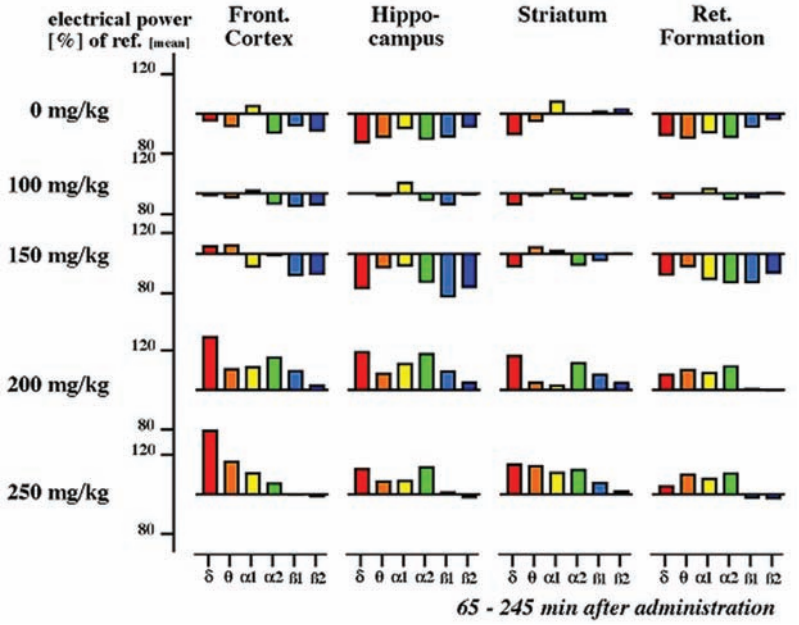

Fig. 2. Dose dependence of frequency changes during the 2nd through 4th hour after administration of Ze 91019 orally. Volume of administration was 1ml/kg. Dosages refer to the valerian siccum extract in the pulverized tablets containing the fixed valerian hop extract combination Ze 91019 solubilized by aid of solutol. Averages are given for n=7-8 animals.

sleep and shortened sleep latency, too [17]. These reports are in line with the present findings in the chronically instrumented, freely moving rats. The extract combination modifies EEG frequencies, which are indicative for prolonged wakefulness and, in addition, may reflect increased sleep pressure.

From the frequency changes in the frontal cortex observed in the present experiments, it can be assumed that cholinergic transmission decreases since increase of delta activity is generally seen after administration of anti-cholinergic drugs like scopolamine, biperidine and methyllycaconitine [18]. The cholinergic neurons scattered throughout the basal forebrain are thought to play an important role in the control of the cortical arousal and the maintenance of wakefulness, although the forebrain histaminergic system and brainstem cholinergic, noradrenergic and serotonergic systems are also involved [7].

Whether the effects seen in the hippocampus after administration of the extract combination might be related to an indirect activation of sleep promoting neurons in the preoptic/anterior hypothalamic area remains to be determined. At least the local network is very close to fulfil such an option [7].

Increases of theta power have been observed after sedation and sleep inducing drugs like clonidine and metedomidine [19], acting at the norepinephrine presynaptic alpha2 receptor. Higher dosages of neuroleptic drugs likewise inducing sedation increased theta activity [20].

In conclusion the present data lend support to the thesis that valerian extract alone and together in the combination with hops mimics the physiological action of adenosine, a somnogenic agent, and therefore may improve falling and staying asleep, both of the actions will increase sleep quality.

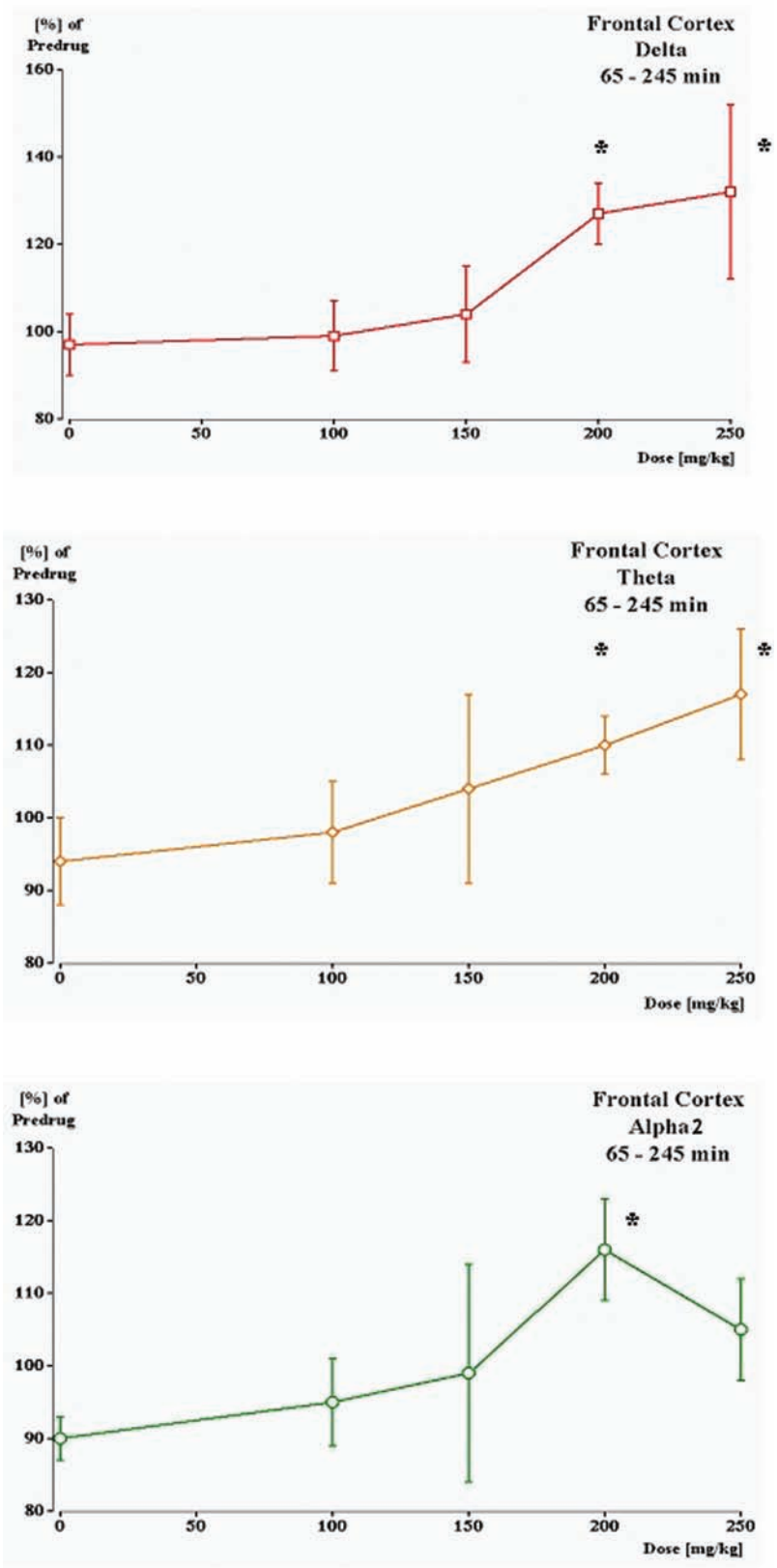

Fig. 3. Dose dependence of single frequency ranges: delta, theta and alpha2 in percent of the pre-drug base line values. Preparation was administered orally. Values are given as mean ± SEM from 7-8 animals ($p < 0.05$).

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