Pharmacological classification of herbal extracts by means of comparison to spectral EEG signatures induced by synthetic drugs in the freely moving rat

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ARTICLE INFO

Article history:
Received 15 February 2013
Received in revised form 16 July 2013
Accepted 16 July 2013
Available online 27 July 2013

Key words:
Herbal extract
Spectral EEG signature
Pharmacological classification
Field potential
In-vivo EEG model

ABSTRACT

Herbal extracts targeting at the brain remain a continuous challenge to pharmacology. Usually, a number of different animal tests have to be performed in order to find a potential clinical use. Due to manifold possible active ingredients biochemical approaches are difficult. A more holistic approach using a neurophysiological technique has been developed earlier in order to characterise synthetic drugs. Stereotactic implantation of four semi-microelectrodes into frontal cortex, hippocampus, striatum and reticular formation of rats allowed continuous wireless monitoring of field potentials (EEG) before and after drug intake. After frequency analysis (Fast Fourier Transformation) electric power was calculated for 6 ranges (delta, theta, alpha1, alpha2, beta1 and beta2). Data from 14 synthetic drugs – tested earlier and representative for different clinical indications – were taken for construction of discriminant functions showing the projection of the frequency patterns in a six-dimensional graph. Quantitative analysis of the EEG frequency pattern from the depth of the brain succeeded in discrimination of drug effects according to their known clinical indication (Dimpfel and Schober, 2003). Extracts from Valerian root, Ginkgo leaves, Paulinia seed, Hop strobile, *Rhodiola rosea* root and *Sideritis scardica* herb were tested now under identical conditions. Classification of these extracts based on the matrix from synthetic drugs revealed that Valerian root and hop induced a pattern reminiscent of physiological sleep. Ginkgo and Paulinia appeared in close neighbourhood of stimulatory drugs like caffeine or to an analgesic profile (tramadol). *Rhodiola* and *Sideritis* developed similar frequency patterns comparable to a psychostimulant drug (methylphenidate) as well as to an antidepressive drug (paroxetine).

1. Introduction

Characterisation of herbal extracts targeting at the brain remains a continuous challenge to pharmacology. Usually, quite a number of different animal tests have to be passed in order to find out in which direction a particular extract might act. Due to several molecular entities or ingredients with possibly different mechanisms of action linear dose-response relationships cannot always be expected. Especially, on a molecular level activation or blockade of multiple receptors by different ingredients make a final drug effect nearly impossible. In addition, prediction of a clinically useful effect based on a particular drug-receptor action remains the exception. Therefore, biochemically based approaches of pharmacological characterisation of herbal drug effects are very difficult and were not really successful in the past. However, the basic communication structure of the brain also involves electric events, which can be measured by neurophysiological techniques. Actions of neurotransmitters on the molecular level result in changes of neuronal ion conductance. These changes of ion conductance determine the activity pattern of a neuron (silence, tonic firing, irregular firing, and burst like activity (Turrigiano et al., 1995). According to Nase et al. (2003) this information on neuronal and synaptic activities is contained within field potentials. By recording of field potentials one can therefore achieve information on local neuronal and synaptic activity.

The question to be solved was: how can we quantify this electric information content and prove the involvement of neurotransmitter activity. In the course of several experimental series agonists and antagonists of particular neurotransmitter receptors were tested by recording field potentials from implanted semi-microelectrodes and wireless transmission from freely moving rats. After frequency analysis of the data by means of Fast Fourier
Transformation (FFT) it was recognised that delta waves (up to 4.5 Hz) were under the control of cholinergic transmission (Dimpfel, 2005) or alpha2 waves (9.75–12.5 Hz) were changed by compounds acting at dopaminergic transmission (Dimpfel, 2008). Frequency pattern changes of a large number of synthetic drugs with known clinical use were fed into a discriminant analysis and led to clustering of drugs with identical clinical use. Using this more holistic approach drugs from 8 clinical indications were successfully separated from each other (Dimpfel and Schober, 2003).

The current experimental series was undertaken to test the possibility that also herbal extracts with known or unknown clinical profiles might induce changes of electric frequency patterns in an analogue manner. If this is the case, herbal extracts might be classified in the same manner, and direct comparison to the pattern produced by synthetic drugs might give information on future use of the extract in humans. Extracts from Valerian root and Hop strobile represent well-known effects with respect to improvement of sleep. Extracts from Ginkgo leaf and Paulinia seed (Guarana) are known for their stimulatory action. These extracts are intended to validate the experimental approach. Extract from Rhodiola rosea root represents a newly defined pharmacological class of so-called adaptogens (Kelly, 2001). Extract from Sideritis scardica herb has not been characterised pharmacologically in-vivo with respect to the brain up to now.

2. Material and methods

Rats were implanted with 4 bipolar concentric steel electrodes within a stereotactic surgical procedure during anaesthesia with Ketamine. All four electrodes were placed 3 mm lateral within the left hemisphere. Dorso-ventral coordinates were 4, 6, 4.2 and 8 mm and anterior coordinates were 3.7, 9.7, 5.7 and 12.2 mm for frontal cortex, striatum, hippocampus, and reticular formation, respectively (according to the atlas of Paxinos and Watson (1982). A pre-constructed 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 by dental cement inter-acting with 3 steel screws placed on distance into the bone. 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 short circuit reference. The base plate was carrying a plug to receive later on the transmitter (weight: 5.2 g including battery, 26 × 12 × 6 mm² of size).

EEG signals were recorded from frontal cortex, hippocampus, striatum and midbrain reticular formation of freely moving rats from inside a totally copper shielded room. Rats were day–night converted (12/12 h) in order to record during the active phase. Signals were wirelessly transmitted by a radio-telemetric system (Rhema Labotechnik, Hofheim, Germany, using 40 Megahertz as carrier frequency) and were amplified and processed as described earlier to give power spectra of 0.25 Hz resolution (Dimpfel et al., 1986, 1988, 1989). 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 predefined 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 min each and displayed on-line. In an off-line procedure spectra were averaged to give longer periods of 30 min or 1 h for further analysis and data presentation.

Statistical evaluation was done by means of non-parametric Wilcoxon, Mann, Whitney-Test. Linear discriminant functions according to Fischer were taken from the results of 14 synthetic drugs with known clinical use tested earlier under identical experimental conditions. Results were depicted as six-dimensional graph with x, y and z space representing results from first three discriminant functions, and colour (RGB mode like in TV) representing the next three discriminant functions. Physiological sleep was recorded during the inactive phase of the rats.

Herbal extracts were kindly provided by the extract companies of the Martin Bauer Group represented by Plantextrakt GmbH & Co. KG, D 91487 Vestenbergsgreuth, Germany as well as Finzelberg GmbH & Co. KG, D 56626 Andernach, Germany.

The characteristic of the six tested herbal extracts are listed in Table 1. All extracts were given orally by gavage dissolved or dispersed in water (1 ml/kg weight). Details of the extracts are given in Table 1. Dosages were chosen by taking into account the human dose recommendation and a relationship factor of 5:1 based on kilogram body weight (Shannon et al., 2007). Synthetic drugs were administered intraperitoneally under otherwise identical experimental conditions. Administration of all preparations were in the presence of an empty stomach. Animals were housed in single cages with food and water ad lib. Maintenance food (Nohrlin 10 H) was achieved from Altromin Spezialfutter GmbH & Co. KG in 32791 Lage, Germany. Animals were held during an inverted light–dark cycle (day=darkness) in order to achieve stable recording during their active phase. The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as testified by authority allowance from Regierungspräsidium Giessen, # 10 0736 540 13 00004 dated 23.02.2010.

3. Results

Extracts from Radix Valerianae and Humulus lupulus were given during the active phase of the animals following the inactive

<table>
<thead>
<tr>
<th>Herb name</th>
<th>Item-no.</th>
<th>Extraction solvent</th>
<th>Drug-extract-ratio native</th>
<th>Content of key substances</th>
<th>Human dose recommended (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radix Valerianae officinalis</td>
<td>0199368</td>
<td>Ethanol 35% V/V</td>
<td>3–6 : 1</td>
<td>0.1–0.2% valerenic acids</td>
<td>600–900</td>
</tr>
<tr>
<td>Ginkgo biloba L. leaves</td>
<td>255530</td>
<td>Water</td>
<td>4.0–4.8:1</td>
<td>0.8–1.2% flavonoids appr. 0.4% Terpene</td>
<td>150–290</td>
</tr>
<tr>
<td>Paulinia cupana Kanth seeds</td>
<td>255497</td>
<td>Ethanol 50% w/w</td>
<td>6.6–8.0:1</td>
<td>9–11% caffeine</td>
<td>100–250</td>
</tr>
<tr>
<td>Humulus lupulus L.</td>
<td>255592</td>
<td>Ethanol 35% v/w</td>
<td>18–22:1</td>
<td>0.1–0.2% 8-prenylnaringenin</td>
<td>120–250</td>
</tr>
<tr>
<td>Radix Rhodiola roseo L.</td>
<td>0550300</td>
<td>Ethanol 70% V/V</td>
<td>3–6:1</td>
<td>NLT 4% xanthohumel</td>
<td>200–400</td>
</tr>
<tr>
<td>Herba Sideritis L. scardica</td>
<td>0232300</td>
<td>Ethanol 20% V/V</td>
<td>5–9:1</td>
<td>NLT 3% rosavinsNLT 1% salidroside</td>
<td>800–1200</td>
</tr>
</tbody>
</table>

Table 1
Details on the herbal extracts used and recommended human dosages. Binomial names are given in cursive letters.
phase. Extract from Valerian root was given orally (by gavage) at a dosage of 60 mg/kg. Despite some increases of spectral power in the frontal cortex during the first hour (not shown) full effect was not seen before the second and third hour (Fig. 1, 2nd line). The pattern as induced by this extract consisted in massive increases of spectral power, mainly in the delta and alpha2 ranges, but also considerably in theta and alpha1 waves. Beta1 waves increased less and beta2 waves were not affected. Strongest effects were seen within the frontal cortex, less in the hippocampus and even less in striatum and reticular formation. This pattern of changes is very reminiscent of changes observed during physiological sleep (Fig. 1, 1st line). Clearly less and more inconsistent changes were observed in the presence of 50 mg/kg of hop strobile (Fig. 1, 3rd line). Again largest effects were seen in the frontal cortex. The anticonvulsive, sedative synthetic drug phenytoin only induces rather small increases of spectral power.

Extracts from Paullinia seed and Ginkgo leaf induced attenuation of spectral power (Fig. 2). Paullinia seed at a dosage of 15 mg/kg led to strong and in comparison to placebo statistically significant attenuation of alpha2 and beta1 waves. But also alpha1 waves were less significant in frontal cortex, hippocampus and striatum but not in the reticular formation (Fig. 2, 2nd line). The spectral changes were nearly identical to those induced by 2.5 mg/kg of caffeine (Fig. 2, 1st line).

Likewise aqueous Ginkgo leaf extract at a dosage of 100 mg/kg induced significant attenuation of alpha2 and beta1 waves in the frontal cortex. Decrease of spectral power was also significant in the striatum but not in the hippocampus or reticular formation (Fig. 2, 3rd line). In the presence of Ginkgo leaf extract also beta2 waves were attenuated significantly except for the reticular formation. The overall pattern of changes was very similar to that observed after administration of 2.5 mg/kg of the synthetic drug tramadol (Fig. 2) and methylphenidate (Fig. 3).

Hydroalcoholic extract of *Rhodiola rosea* root at a dosage of 100 mg/kg induced statistically significant attenuation of alpha2 and beta1 waves in all brain areas (Fig. 3, 2nd line). In addition, spectral theta power was diminished in frontal cortex, hippocampus and reticular formation. Only within the frontal cortex delta power was attenuated. The overall pattern somewhat resembled those changes as observed in the presence of methylphenidate (Fig. 3) and the antidepressant synthetic drug paroxetine (Fig. 3, 4th line).

Extract of *Sideritis scardica* herb at a dosage of 100 mg/kg produced spectral changes, which were similar to those observed in the presence of *Rhodiola rosea* root extract at a dosage of 100 mg/kg. Again main effects were seen with regard to significant attenuation of alpha2 waves followed by decreases in spectral theta power in the frontal cortex and hippocampus. Some similarity was found to spectral changes seen after the administration of 1 mg/kg paroxetine, a synthetic antidepressive drug (Fig. 3, 4th line) and methylphenidate (Fig. 3, 1st line).

These comparisons of the effects of herbal extracts with synthetic drugs tested under identical conditions relay on our subjective impression. In order to get clear quantitative evaluation of the pattern changes statistical tools are necessary. One possibility consists in using linear discriminant analysis. After having established a set of discriminant functions on the base of earlier testing of synthetic drugs with different clinical indications, this
matrix of discriminant functions was used to characterise these 6 herbal extracts. Twenty-four variables (6 frequency ranges times 4 brain regions) were taken for analysis. Results from the first three discriminant functions are depicted in space (x, y and z axis). Next results from further 3 discriminant functions are documented using the so-called RGB mode (additive colour mixture like used in TV). Thus, compounds projected into close neighbourhood signalise a similar clinical use. If they show the same colour their mechanism of action seems to be in common. The result is shown in Fig. 4.

As one can see in this projection hop strobile and Valerian root extract position near physiological sleep. Extracts from Paullinia seed and Ginkgo leaf are found near the synthetic drug methylphenidate and caffeine. Extracts from Rhodiola rosea root and Sideritis scardica herb are seen very close to tramadol and not too far away from paroxetine. Thus, subjective impression of induction of analogues spectral signatures of herbal drugs in rats in comparison to the pharmacological effects of synthetic drugs is confirmed by use of discriminant analysis for statistical evaluation.

4. Discussion

The holistic approach of testing drugs and herbal preparations by using neurophysiological methods provides information on drug effects with respect to an intermediate anatomical level. Since it is not easily possible to follow drug-induced changes of brain activity on a molecular level in vivo (i.e. drug-receptor interactions), the anatomically higher integrative level of focal field potentials provides information half way to behaviour. The method has been validated by earlier testing of particular reference drugs with well-known clinical actions and filed indications to treat different diseases. This matrix of discriminant functions produced by clinically identified drug actions has now been used to scan for plant-derived extracts with possibly similar pharmacological features.

Comparison of spectral signatures of the field potentials recorded from the depth of the brain revealed that extracts from Valerian root and Hop strobile induced frequency changes reminiscent to those observed during natural sleep. This can be regarded as a certain piece of evidence that these extracts induce healthy sleep. On the opposite, the effect of diazepam (and other benzodiazepines or barbiturates tested earlier) must be regarded more as narcotics, which – at lower dosages – induce sedation but not healthy sleep. They also produce more unwanted side effects. A systematic review and meta-analysis of the effects of Valerian root extract on sleep stated that the available evidence suggests that Valerian root extract might improve sleep quality without producing side effects (Bent et al., 2006). This was confirmed in a newer study also in combination with hops (Dimpfel and Suter, 2008). Also for hops alone sedating effects have been described in the literature (Schiller et al., 2006; Zanoli and Zavatti, 2008). Further evidence for sedative effects comes from the ability of hops to enhance pentobarbital sleep time (Zanoli et al., 2005). Thus, positioning of valerian and hops extract in the neighbourhood of natural sleep suggests plausible classification by feeding the spectral signatures of both into discriminant analysis.

Further evidence for a meaningful classification of herbal preparations by quantitative measurement of field potentials comes from testing extracts of Paullinia seed and Ginkgo leaf.
Paullinia seed contains some 8–10% of caffeine. Accordingly, the projection of its spectral signatures by discriminant analysis shows Paullinia seed extract in the neighbourhood of caffeine and with the same colour. However, the pattern of changes is not absolute identical to that of caffeine since extract from Paullinia seed produced stronger attenuation of alpha1 waves in the hippocampus and striatum. Also, beta1 power decreased stronger in relation to alpha2 power. Other evidence for some differences between the effect of caffeine and Paullinia seed extract was also reported in mouse behaviour using higher dosages of both (Campos et al., 2005). In humans improved cognitive performance in the presence of Paullinia seed extract was reported (Kennedy et al., 2004). Interestingly, the spectral signature of the tested Ginkgo leaf extract (water as extraction solvent, low enrichment of actives) resembles very much the one observed in the presence of methylphenidate, a drug used to treat attention-deficit hyperactivity disorder (ADHD). However, a double blind, randomized clinical trial revealed that Ginkgo leaf (high enriched special extract) was also reported in mouse behaviour using higher dosages of both (Campos et al., 2005). In humans improved cognitive performance in the presence of Paullinia seed extract was reported (Kennedy et al., 2004).

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where Sideritis scardica herb extracts inhibit reuptake of serotonin, dopamine and noradrenaline (Knörlé, 2012). The similarity of the spectral signature of Sideritis scardica herb extract to the effects of Ginkgo leaf extract are in line with data showing enhancement of long term potentiation in the hippocampus slice preparation in vitro for both extracts (unpublished results). Further experiments have to be done in order to learn more about this exciting possibility.

In summary, the holistic approach of finding spectral signatures of telemetrically transmitted field potentials from freely moving rats might open the door to more specific recognition of potential use of plant-derived preparations in humans. There is quite a bit of evidence that analogous spectral signatures can be found in the human EEG in the presence of psychoactive drugs (i.e. diazepam, haloperidol) suggesting to use quantitative EEG in humans to follow time and dose dependent actions of plant derived extracts. Clinical results were obtained using source density EEG brain mapping with CATEEM® in the presence of a combination of Valerian and hops (Vonderheide-Guth et al., 2000) and a lozenge containing a mixture of hops, lemon balm and oat (Dimpfel et al., 2004).

References

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Fig. 4. Comparison of different plant derived extracts to the spectral frequency patterns induced by conventional synthetic drugs with known clinical effects tested under identical conditions by means of discriminant analysis using the “matrix” of discriminant functions established by synthetic drugs. Please note that extracts from Rhodiola rosea root and Sideritis scardica herb position near to Tramadol, an analgesic drug, but the colour produced by Sideritis scardica herb extract goes into the direction of Ginkgo leaf extract.


