Journal of Ethnopharmacology 149 (2013) 583–589

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

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

ABSTARCT

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 possibly 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, Paullinia 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 Paullinia 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 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 prediction of the 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

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http://dx.doi.org/10.1016/j.jep.2013.07.029

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 Paullinia 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 interacting 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, $26 \times 12 \times 6$ mm^3 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 Labortechnik, 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 by 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 in account the human dose recommendation and a relationship factor of 5–10:1 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 stabile 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 1
Details on the herbal extracts used and recommended human dosages. Binomial names are given in cursive letters.

Herb name	Item-no.	Extraction solvent	Drug-extract-ratio native	Content of key substances	Human dose recommended (mg)
Radix *Valerianae officinalis*	0199368	Ethanol 35% V/V	3–6 : 1	0.1–0.2% valerenic acids	600–900
Ginkgo biloba L. leaves	255530	Water	4.0–4.8:1	0.8–1.2% flavonoids appr. 0.4% Terpene	150–290
Paullinia cupana Kunth seeds	255497	Ethanol 50% w/w	6.6–8.0:1	9–11% caffeine	100–250
Humulus lupulus L.	255592	Ethanol 55% w/w	18–22:1	0.1–0.2% 8-prenylnaringenin NLT 4% xanthohumol	120–250
Radix *Rhodiola rosea* L.	0550300	Ethanol 70% V/V	3–6:1	NLT 3% rosavinsNLT 1% salidroside	200–400
Herba *Sideritis* L. scardica	0232300	Ethanol 20% V/V	5–9:1	0.5–1.5% flavonoids 0.1–0.4% caffeoylquinic acids	800–1200