Phytomedicine 19 (2012) 334–340

Contents lists available at SciVerse ScienceDirect

Phytomedicine

journal homepage: www.elsevier.de/phymed

$GABA_A$ receptor modulators from Chinese herbal medicines traditionally applied against insomnia and anxiety

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ARTICLE INFO

Keywords:
Atractylodes macrocephala
$GABA_A$ receptors
Sesquiterpene lactones
Atractylenolide
Voltage clamp
Positive allosteric modulation

ABSTRACT

Several Chinese herbal medicines (CHMs) are used in the treatment of insomnia, restlessness, or anxiety. However, mechanisms underlying this effect and scientific proof for their traditional use is scarce. In the present study CHMs were screened for their ability to modulate GABA-induced chloride currents (I_{GABA}), and active principles were isolated thus providing scientific evidence for their use as sedative and/or anxiolytic agents in CM. Herbal drugs were extracted successively with petroleum ether, ethyl acetate, methanol and water and further fractionated according to their bioactivity. The obtained extracts, fractions and finally pure compounds were tested for their ability to potentiate I_{GABA} using the two-microelectrode voltage clamp technique on recombinant $\alpha_1\beta_2\gamma_{2S}$ $GABA_A$ receptors expressed in *Xenopus laevis* oocytes.

From all tested extracts the petroleum ether extract of *Atractylodes macrocephala* Koidz. rhizomes showed the strongest I_{GABA} potentiation and was studied in more detail.

This led to the isolation of the main components atractylenolide II and III, which seem to be responsible for the observed positive modulation of I_{GABA} ($166 \pm 12\%$, $n = 3$ and $155 \pm 12\%$, $n = 3$, respectively) *in vitro*. They were more active than the analogous compound atractylenolide I ($96 \pm 3\%$, $n = 3$) which differs in an additional double binding in position 9, 9a. Furthermore it could be shown that this effect is mediated independently of the benzodiazepine (BZ) binding site.

In conclusion, *A. macrocephala* exerts its *in vitro* activity on recombinant $GABA_A$ receptors mainly through the two sesquiterpene lactones atractylenolide II and III (Fig. 1). This positive allosteric modulation of I_{GABA} may partially be responsible for the traditional ethnopharmacological use of this herbal drug as a sedative.

Introduction

In Chinese medicine (CM) several herbal drugs are applied in the treatment of insomnia or anxiety disorders, two highly prevalent mental disorders (Somers et al. 2006; Roth 2007). Although such herbal remedies are frequently used and considered effective and well-tolerated, studies confirming their ethnopharmacological use are often lacking. From the 12 Chinese herbal medicines (CHMs), which are listed in Table 1, we selected for our study, only some have been investigated for anxiolytic, sedative or other $GABA_A$ receptor related effects *in vivo*: sedative effects for flavonoids isolated from *Albizia julibrissin* flowers (Kang et al. 2000) and anxiolytic effects through *Albizia* bark extracts have been already published (Kim et al. 2004). Furthermore, the alkaloid hormone isolated from *Tribulus terrestris* is supposed to be mildly sedating and affect the locomotor activity in sheep (Bourke et al. 1992). *Nelumbo nucifera* seed embryos contain isoquinoline alkaloids like neferine and related constituents, which seem to have anxiolytic and sedative activity *in vivo* (Sugimoto et al. 2008). From *Dimocarpus longan*, adenosine was determined as the active anxiolytic principle in a bioactivity guided approach using the Vogel Conflict Test (Okuyama et al. 1999) and sedative-hypnotic activity was published for a decoction from the stem of *Polygonum multiflorum* (Yang et al. 1990). In search for the molecular mechanism underlying these traditional sleep-remedies, they were investigated for their ability to modulate the γ-aminobutyric acid (GABA) type A ($GABA_A$) receptor. GABA is the major inhibitory neurotransmitter in the mammalian brain and hence the $GABA_A$ receptor represents a viable target when searching for anxiolytic or sedative

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doi:10.1016/j.phymed.2011.10.009

Table 1
Selected 12 TCM herbal drugs for screening on recombinant $\alpha_1\beta_2\gamma_{2S}$ $GABA_A$ receptor expressed in *X. laevis* oocytes. Footnotes indicate literature where the respective plant is mentioned as a sedative and/or anxiolytic agent. The extract-induced potentiation of I_{GABA} is given as mean ± SEM from at least three oocytes from two different batches, whereby the extracts were always tested in concentrations of 100 μg/ml.

Abb.	Plant name	Drug	Pinyin	Potentiation of I_{GABA} ± SEM (%)			
				PE	EtOAc	MeOH	Water
AC	[a]*Alibizia julibrissin* Durazz. (Fabaceae)	Cortex	hé huān pí	18 ± 4	12 ± 6	7 ± 3	−9 ± 5
AF	[a]*Alibizia julibrissin* Durazz. (Fabaceae)	Flos	hé huān huā	11 ± 11	1 ± 8	0 ± 5	2 ± 6
AR	[a]*Arisaema erubescence* (Wall.) Schott.; *A. heterophyllum* BL.; *A. amurense* Maxim. (Araceae)	Rhizoma	tiān nán xīng	−3 ± 9	−2 ± 3	1 ± 3	−25 ± 8
AE	*Arnebia euchroma* (Royle) Johnst., *Lithospermum erythrorhizon* Sieb. et Zucc.	Rhizoma	zǐ cǎo	44 ± 13	21 ± 5	3 ± 3	−8 ± 10
AM	[a]*Atractylodes macrocephala* Koidz.	Rhizoma	bái zhú	322 ± 48	194 ± 78	76 ± 10	22 ± 14
DL	[b]*Dimocarpus (Euphoria) longan* Lour. (Sapindaceae)	Arillus	long yǎn ròu	6 ± 3	−6 ± 1	8 ± 5	13 ± 5
FS	[b]*Forsythia suspensa* (Thunb.) Val. (Oleaceae)	Fructus	lián qiào	92 ± 34	11 ± 2	7 ± 8	−19 ± 3
LB	[a]*Lilium brownii* F.E. Brown *var. viridulum* Baker W; *L. lancifolium* Thunb., *L. pumilum* DC. (Liliaceae)	Bulbus	bǎi hé	36 ± 8	95 ± 25	85 ± 6	4 ± 13
LG	[b]*Lophaterum gracile* Brong. (Gramineae)	Herba	dàn zhú yè	6 ± 2	6 ± 6	−5 ± 14	−17 ± 6
NN	[c]*Nelumbo nucifera* Gaertn. (Nymphaeaceae)	Plumula	lián zǐ xīn	−9 ± 5	−12 ± 26	−16 ± 8	−10 ± 4
PM	[a]*Polygonum multiflorum* Thunb. (Polygonaceae)	Caulis	yè jiāo téng	33 ± 16	−2 ± 15	−1 ± 8	−2 ± 7
TT	[d]*Tribulus terrestris* L. (Zygophyllaceae)	Fructus	cì jí lí	−44 ± 14	−5 ± 6	7 ± 8	14 ± 2

[a] In the case of *Arisaematis rhizome* prep. we refer to its use against convulsions since anti-epileptic agents can target the $GABA_A$ receptor (Bensky et al., 2006).
[b] Hempen (2007).
[c] Sugimoto et al. (2008).
[d] Huang (1999).

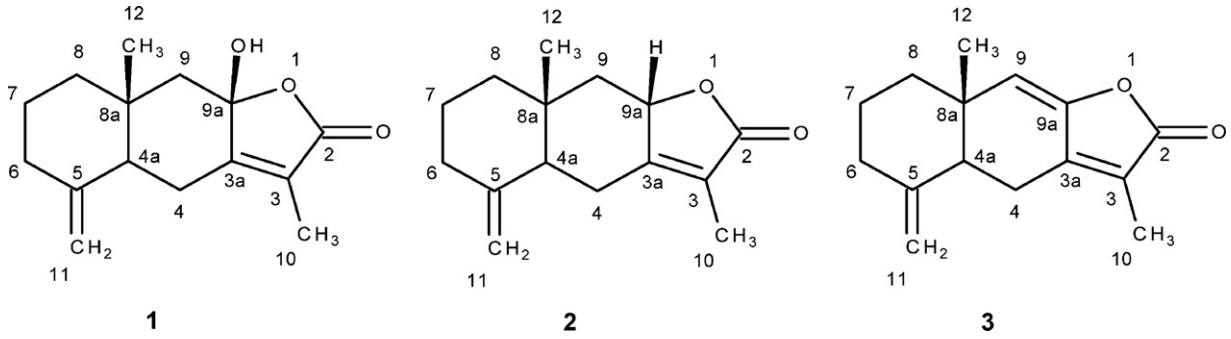

Fig. 1. Chemical structures of the sesquiterpene lactones atractylenolide III (1), atractylenolide II (2) and atractylenolide I (3). While in atractylenolide III and II a hydroxyl or proton is positioned on C8, atractylenolide I bears an additional double bound in position 9 and 9a.

components from natural origin (Johnston et al. 2006; Khom et al. 2007; Trauner et al. 2008). The $GABA_A$ receptor itself is a heteropentameric ligand gated ion channel responsible for fast inhibition of neurotransmission (Mody and Pearce 2004), whereby from many possible receptor subtypes the $\alpha_1\beta_2\gamma_{2S}$ receptor is the most abundant (Moehler 2006; Olsen and Sieghart 2009). In the present study effects of different CHM extracts, fractions and isolated compounds on GABA-induced chloride currents (I_{GABA}) were investigated using the two-microelectrode voltage clamp technique and an automated fast perfusion system on *Xenopus laevis* oocytes expressing $\alpha_1\beta_2\gamma_{2S}$ $GABA_A$ receptors. Since the petroleum ether extract of bái zhú (rhizomes of *Atractylodes macrocephala* Koidz.) displayed highest activity it was further investigated using a bioactivity guided approach. It was found that the sesquiterpene lactones atractylenolide II and III (Fig. 1) are the active principles of bái zhú, which potentiated I_{GABA} concentration dependently and independent of the benzodiazepine binding site.

Materials

Chemicals

For HPLC and crystallization analytical grade solvents were used. For extraction and isolation on silica gel columns solvents of highest available purity were used (VWR, Vienna, Austria).

Diazepam, flumazenil and ND96 reagents were purchased from Sigma (Vienna, Austria).

Plant materials

Herbal drugs were purchased from Plantasia (Oberndorf, Austria). Voucher specimens are deposited at the Department of Pharmacognosy, University of Vienna.

Extraction

The ground drugs were soaked in 500 ml petroleum ether for 10 min, and extracted on the water bath under reflux for 30 min. The obtained extracts were filtered and evaporated to dryness. The remaining drug material was air-dried overnight and extracted with each 500 mL of ethyl acetate (EtOAc), MeOH and distilled water likewise, whereby the water extract was lyophilized.

Fractionation by semi-preparative HPLC

HPLC activity profiling was carried out with a Shimadzu instrument consisting of two LC-8A pumps, a SIL-10AP autosampler, a SPD-M20A diode array detector, a FRC-10A fraction collector and a CBM-20A interface. A Nucleosil 100-7, C-18 (250 mm × 21 mm, 5 μm) column from Machery-Nagel (Düren,