European Journal of Pharmacology 668 (2011) 57–64

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Molecular and Cellular Pharmacology

Insights into structure–activity relationship of $GABA_A$ receptor modulating coumarins and furanocoumarins

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ARTICLE INFO

Article history:
Received 14 December 2010
Received in revised form 20 June 2011
Accepted 24 June 2011
Available online 5 July 2011

Keywords:
$GABA_A$ receptor
(Furano)-coumarin
Osthole
Oxypeucedanin
Voltage-clamp technique
Pharmacophore model

ABSTRACT

The coumarins imperatorin and osthole are known to exert anticonvulsant activity. We have therefore analyzed the modulation of GABA-induced chloride currents (I_{GABA}) by a selection of 18 coumarin derivatives on recombinant $\alpha_1\beta_2\gamma_{2S}$ $GABA_A$ receptors expressed in *Xenopus laevis* oocytes by means of the two-microelectrode voltage clamp technique. Osthole ($EC_{50} = 14 \pm 1$ μM) and oxypeucedanin ($EC_{50} = 25 \pm 8$ μM) displayed the highest efficiency with I_{GABA} potentiation of 116 ± 4% and 547 ± 56%, respectively. I_{GABA} enhancement by osthole and oxypeucedanin was not inhibited by flumazenil (1 μM) indicating an interaction with a binding site distinct from the benzodiazepine binding site. In general, prenyl residues are essential for the positive modulatory activity, while longer side chains or bulkier residues (e.g. geranyl residues) diminish I_{GABA} modulation. Generation of a binary classification tree revealed the importance of polarisability, which is sufficient to distinguish actives from inactives. A 4-point pharmacophore model based on oxypeucedanin – comprising three hydrophobic and one aromatic feature – identified 6 out of 7 actives as hits. In summary, (oxy-)prenylated coumarin derivatives from natural origin represent new $GABA_A$ receptor modulators.

1. Introduction

The gamma-aminobutyric acid type A receptor ($GABA_A$) is a ligand gated ion channel mediating fast inhibition of neuronal signal transmission (Mody and Pearce, 2004). Binding of GABA to $GABA_A$ receptors induces hyperpolarization of the neuronal membrane due to an increased chloride influx and thus decreases or inhibits ongoing neurotransmission. The $GABA_A$ receptors are heteropentameric proteins, which can assembly from 19 different subunits: α_{1-6}, β_{1-3}, γ_{1-3}, δ, π, ε, θ, and ρ_{1-3} and potentially generate a large variety of receptor subtypes (Simon et al., 2004). From theoretically over 150.000 possible $GABA_A$ receptors only a few seem to occur *in vivo* in the mammalian central nervous system (Olsen and Sieghart, 2009). The most abundant receptor subtype consists of 2 α_1, 2 β_2 and 1 $\gamma_{2S/L}$ subunit (McKernan and Whiting, 1996; Sieghart and Sperk, 2002). While binding of GABA opens $GABA_A$ receptor channels, there is also evidence for binding sites interacting with benzodiazepines, general anesthetics, barbiturates and many other therapeutically important drugs (Korpi et al., 2002; Sieghart, 1995; Sieghart and Enna, 2006). In addition to drugs that are in clinical use a variety of structurally diverse natural products have been shown to elicit positive modulatory effects on $GABA_A$ receptors, e.g. borneol (Granger et al., 2005), thymol (Priestley et al., 2003), valerenic acid (Khom et al., 2007; Trauner et al., 2008), piperin (Zaugg et al., 2010), flavonoids (Fernandez et al., 2008; Huen et al., 2003), polyacetylenes (Baur et al., 2005), and various others (Johnston et al., 2006).

Compared to other natural compound classes like flavonoids or monoterpenes, the action of coumarins on $GABA_A$ receptors is largely unknown. However, coumarins often occur in plants that are used as sedatives or spasmolytical agents in traditional medicinal systems worldwide (Murray et al., 1982; O'Kennedy and Thorne, 1997). Furthermore, *in vivo* antiepileptic activity of coumarins was reported by Luszczki and co-workers (Luszczki et al., 2007a,b, 2009a,b).

Evidence for interaction of coumarins with $GABA_A$ receptors comes also from binding studies suggesting that phellopterin and imperatorin interact with the benzodiazepine binding site of the $GABA_A$ receptor (Bergendorff et al., 1997; Dekermendjian et al., 1996).

In the present study we examine the effects of 18 (furano-) coumarins on chloride currents (I_{GABA}) through recombinant $\alpha_1\beta_2\gamma_{2S}$ $GABA_A$ receptors expressed in *Xenopus laevis* oocytes and provide first insights into the structural requirement for a positive modulatory effect.

2. Material and methods

2.1. Chemicals and substances

γ-Amino butyric acid (GABA), reagents for ND96 solution, diazepam and flumazenil were purchased from Sigma (Vienna, Austria).

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doi:10.1016/j.ejphar.2011.06.034

Bergamottin, bergapten, bergaptol, coumarin, isobergapten, isopimpinellin, scopoletin and umbelliferone were purchased from Extrasynthese (Lyon, France). Auraptene and isoimperatorin were purchased from LGC Standards (Wesel, Germany). Oxypeucedanin was purchased from Phytolab (Vestenbergsreuth, Germany). Phellopterin was purchased from Sequoia Research Products Ltd. (Pangbourne, UK). Pimpinellin was purchased from Herboreal Ltd. (Edinburgh, UK). Ostruthin (purity ≥ 98%) and ostruthol (purity ≥ 98%) were isolated from *Peucedanum ostruthium* L. (Koch) by Vogl et al. (2011) and imperatorin and osthole were isolated from *Cnidium monnieri* L. as follows: a petroleum ether extract of Cnidium monnieri fruits was first subjected to semi-preparative HPLC using a RP-18 column (Nucleosil 100, Machery-Nagel) and a gradient elution consisting of water (solvent A) and acetonitril (solvent B) with a concentration of B of 35% B for 15 min, followed by an increase of B to 80% in 5 min and a steady concentration of B for 7 min followed by a decrease to 35% B in 3 min. Flow rate was set at 27.6 mL/min. Fraction 16, which according to literature contains imperatorin and osthole, was subjected to normal phase column chromatography on silica gel (60 x 0.5 cm i.d.) using n-hexane:EtOAc (95:5) as mobile phase (flow rate 10 mL/h, fraction volume: 5 mL). Fractions were screened by TLC on silica gel coated aluminum plates KG60 F254 (Merck, Germany) using n-hexane:EtOAc (90:10) as mobile phase. Fractions 25–36 (blue fluorescent zone in the TLC screening) and fractions 46–61 (brown fluorescent spot in the TLC screening) were unified to yield two cumulative fractions. Their structure was elucidated by 1- and 2-D 1H and 13 C-NMR as imperatorin (purity: ≥98%) and osthole (purity: ≥97%), respectively. Purity was determined using HPLC by comparing UV spectra and retention time to reference substances which were purchased from Sigma (Vienna, Austria).

2.2. Voltage clamp and fast solution exchange on Xenopus oocytes

Preperation of stage V–VI oocytes from *Xenopus laevis* (NASCO, USA) and injection of cRNA were done as previously described (Khom et al., 2006). Female frogs were anesthetised 15 min prior to surgery using 0.2% solution of MS-222 (Sigma, Vienna, Austria) and parts of the ovaries were removed. Remaining follicle membranes were enzymatically digested with 2 mg/mL collagenase Type 1 A (Sigma, Vienna, Austria). Synthesis of capped off run-off poly (A+) cRNA transcripts was performed from linearized cDNA templates (pCMV vector). cRNAs were diluted with DEPC-treated water and stored at −80 °C. Injection of 10–50 nL of the different cRNA solutions was carried out on the day of isolation. To ensure the expression of the γ-subunit, cRNAs of α_1, β_2, and γ_{2S} were injected in a ratio of 1:1:10 (Baburin et al., 2008; Boileau et al., 2003). Successful expression of the γ-subunit was determined by application of diazepam (300 nM). Injected oocytes were stored at 18 °C in penicillin and streptomycin supplemented ND96 solution, containing 96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$*$6H_2O$, 1.8 mM $CaCl_2$ and 5 mM HEPES (pH 7.4) in double distilled water.

Chloride currents through $GABA_A$ receptors were measured by means of the two-microelectrode voltage clamp method making use of a TURBO TEC 03X amplifier (npi electronic, Tamm, Germany) at a holding potential of −70 mV as previously described (Baburin et al., 2006). Current measurements were recorded with pCLAMP 10 data acquisition software (Molecular Devices, Sunnyvale, CA, USA). ND96 was used as bath solution. Microelectrodes (Harvard Apparatus, Kent, UK) with resistances between 1 and 3 MΩ were pulled by means of a microelectrode puller (Narashige, Tokyo, Japan) and filled with 2 M KCl.

GABA and compounds were applied to oocytes by means of the ScreeningTool (npi electronic, Tamm, Germany) fast perfusion system as described by Baburin et al. (2006). Stock solutions of the tested compounds (100 mM) were prepared in DMSO and stored at −20 °C. GABA and test solutions were prepared freshly every day. The DMSO concentration of 1% in both, the control and test solutions, did not affect GABA-induced chloride current (I_{GABA}). In the DMSO-stock solutions (10 mM) and the aqueous test solutions used no precipitates or turbidity was observed and thus the compounds were regarded as fully dissolved.

I_{GABA} modulation was measured at a GABA concentration eliciting between 5 and 10% of the maximal current amplitude (EC_{5-10}), corresponding to 3–10 μM GABA. The EC_{5-10} was established at the beginning of each experiment. In the presence of compound concentrations higher than 30 μM wash out periods were extended to up to 10 min to exclude effects of receptor desensitization on current amplitudes.

2.3. Data analysis

Compound induced changes in chloride current amplitudes were calculated as $I_{(GABA+compound)}/I_{GABA} - 1$, where $I_{(GABA+compound)}$ is the current response in the presence of a given compound and I_{GABA} is the control GABA current.

Concentration–response curves were generated and the data were fitted by nonlinear regression analysis using Origin Software (OriginLab Corporation, Northampton, MA, US). Data were fitted to the equation $1/(1+(EC_{50}/[compound]^{nH})$, where EC_{50} is the

Table 1
Class labels and selected physicochemical descriptors of the (furano-)coumarins including mean potentiation of I_{GABA} by selected coumarin derivatives (100 μM). 7 compounds with an I_{GABA} potentiation above 20% were classified as active (1) while the other 11 components were regarded as inactive (0).

Compound	Potentiation (%)	Class	apol	a_acc	a_don	b_1rotN	logP(o/w)	mr	TPSA
Coumarin	1,5	0	21,44	1	0	0	2,18	4,14	26,30
Umbelliferone	−4,4	0	22,25	2	1	0	1,90	4,26	46,53
Scopoletin	−1,8	0	26,14	3	1	1	1,90	4,90	55,76
Osthole	124,5	1	39,47	2	0	3	3,20	7,05	35,53
Auraptene	−0,1	0	50,52	2	0	6	3,85	8,85	35,53
Ostruthin	2,6	0	50,52	2	1	5	3,88	8,81	46,53
Bergaptol	−19,9	0	26,56	2	1	0	2,17	5,16	59,67
Isopimpinellin	6,3	0	33,56	3	0	2	2,35	6,31	57,90
Bergapten	−10,5	0	29,66	2	0	1	2,43	5,68	48,67
Isoimperatorin	33,8	1	40,70	2	0	3	3,25	7,49	48,67
Imperatorin	54,1	1	40,70	2	0	3	3,25	7,49	48,67
Phellopterin	56,5	1	44,60	3	0	4	3,16	8,13	57,90
Heraclenin	32,9	1	41,51	3	0	3	2,99	7,45	61,20
Oxypeucedanin	550	1	41,51	3	0	3	2,99	7,45	61,20
Bergamottin	−7,2	0	54,84	2	0	6	4,11	9,77	48,67
Ostruthol	−6,6	0	57,24	4	1	6	3,06	10,15	95,20
Isobergapten	4,6	0	29,66	2	0	1	2,43	5,68	48,67
Pimpinellin	65,4	1	33,56	3	0	2	2,10	6,31	57,90