UC Irvine

UC Irvine Previously Published Works

Title

Hydroxycinnamic acid amides from *Scopolia tangutica* inhibit the activity of M1 muscarinic acetylcholine receptor in vitro.

Permalink

<https://escholarship.org/uc/item/085072zx>

Authors

Zhang, Yan
Long, Zhen
Guo, Zhimou
et al.

Publication Date

2016

DOI

10.1016/j.fitote.2015.11.007

Peer reviewed

Hydroxycinnamic acid amides from *Scopolia tangutica* inhibit the activity of M1 muscarinic acetylcholine receptor in vitro

Yan Zhang^{a,*}, Zhen Long^{b,1}, Zhimou Guo^b, Zhiwei Wang^c, Xiuli Zhang^b, Richard D. Ye^a, Xinmiao Liang^b, Olivier Civelli^c

^a School of Pharmacy, Shanghai Jiao Tong University, Shanghai 200240, People's Republic of China

^b Key laboratory of Separation Science for Analytical Chemistry, Key Lab of Natural Medicine, Liaoning Province, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, People's Republic of China

^c Department of Pharmacology, University of California, Irvine, CA 92697, United States

ARTICLE INFO

Article history:

Received 15 September 2015

Received in revised form 12 November 2015

Accepted 13 November 2015

Available online 14 November 2015

Keywords:

Scopolia tangutica Maxim

Hydroxycinnamic acid amides

M1 muscarinic acetylcholine receptor

ABSTRACT

Scopolia tangutica Maxim (*S. tangutica*) extracts have been traditionally used as antispasmodic, sedative, and analgesic agents in Tibet and in the Qinghai province of China. Their active compositions are however poorly understood. We have recently isolated five new hydroxycinnamic acid (HCA) amides along with two known HCA amides, one cinnamic acid amide from these extracts. In this study, we evaluate their abilities to inhibit carbachol-induced activity of M1 muscarinic acetylcholine receptor along with the crude extracts. Chinese hamster ovary cells stably expressing the recombinant human M1 receptor (CHO-M1 cells) were employed to evaluate the anticholinergic potentials. Intracellular Ca^{2+} changes were monitored using the FLIPR system. Five HCA amides as well as the crude *S. tangutica* extract displayed dose-dependent inhibitory effects against M1 receptor. These findings demonstrate that HCA amides are part of the M1 receptor-inhibiting principles of *S. tangutica*. Since blockade of parasympathetic nerve impulse transmission through the inhibition of the M1 receptor lessens smooth muscle spasms, our findings provided a molecular explanation for the traditional use of *S. tangutica* against spasm.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Scopolia tangutica Maxim (*S. tangutica*) is one of the traditional Chinese medicines used in Tibet and in the Qinghai province of West China. Its extracts have been traditionally used as antispasmodic, sedative, and analgesic agents [1,2]. Its medicinal importance is associated with high biologically active tropane alkaloids, in particular anisodine and scopolamine [3–5]. These alkaloids primarily affect the parasympathetic nervous system and act as muscarinic acetylcholine receptor (mAChR) antagonists [6–8]. mAChR antagonists block the transmission of parasympathetic nerve impulses by acetylcholine and consequently lessen the spasms of smooth muscle [9,10]. Recently we have isolated five new hydroxycinnamic acid (HCA) amides along with two known HCA amides, one cinnamic acid amide from *S. tangutica* [11,12]. The conjugates of HCAs with polyamines have been found in a wide variety of plants and are concentrated in the reproductive organs of many higher plants [13–15]. The HCA-spermidine conjugates have been proven to be useful for chemotaxonomic purposes in several plant

families [16–18]. The HCA amides have been proposed to have a number of biological activities, including the effects of anti-fungi, anti-bacteria and anti-tumor [19–21], suggesting that HCA amides may have great potential for identifying new molecules for different diseases. It is interesting to note the similarity of the plant derived HCA amides to polyamine conjugates found in the invertebrate venom [22,23]. Some of these invertebrate toxins have been found as neurotransmitter receptor ligands [24,25]. In this study, we examined the anticholinergic potential of these HCA amides and compared the anticholinergic properties to the crude extract. The goal of the study is to foster a better understanding of the traditional use of *S. tangutica* and pursuit of the discovery of novel drugs.

mAChRs belong to G protein-coupled receptors (GPCR) family A. They are found in nervous system where they mediate the metabotropic actions of the neurotransmitter acetylcholine, in particular its parasympathetic action. They are major drug targets in human disease therapies. Five subtypes of mAChRs, classified as M1–M5, have been identified [26]. M1, M3 and M5 receptors couple to Gq proteins, which mediate intracellular calcium mobilization upon stimulation. M2 and M4 receptors couple to Gi proteins which inhibit adenylyl cyclase activity [27]. The sequence similarities found among these receptors infer that binding of a ligand to one of them should insure binding to all. In this study we chose to use the M1 receptor as the lead receptor.

* Corresponding author.

E-mail address: zhangyan_sjtu@sjtu.edu.cn (Y. Zhang).

¹ These authors contributed as co-first authors.

2. Materials and methods

2.1. Plant materials

The roots of *S. tangutica* were collected from Jiuzhi county (at 100°40'19" E; 33°16'56" N), Qinghai province, People's Republic of China. Voucher specimens (QTPMB) were deposited at Qinghai–Tibet Plateau Museum of Biology, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, and were identified by Senior Engineer Lijuan Mei.

2.2. Extract preparation and compound purification

The crude extract was prepared as described before [28]. In brief, 95 g *S. tangutica* was grounded into powder and extracted twice with 1 L ethanol–water (95:5, v/v) at 80 °C for 3 h. The crude extracts were obtained by rotary evaporation of the solvent. 1 mL of crude extract was dissolved in 5 mL of methanol. An aliquot of 0.1 mL of crude extract sample was applied to the conditioned SCX-based solid phase extraction column. The isolation and identification of pure compounds from *S. tangutica* are extensively described by our most recent report [12]. Briefly, *S. tangutica* were extracted by 95% ethanol. The isolation of pure compounds was achieved by multi-dimensional HPLC. The identity of the isolated compounds was confirmed by spectroscopic and spectrometric methods (1D- and 2D-NMR, LC–MS, optical rotation). The purity of all isolated compounds was ≥98%.

2.3. M1 receptor activity measurement

Chinese hamster ovary cells stably expressing the recombinant human M1 receptor (CHO-M1 cells) were kindly provided by Dr. Naoto Hoshi (University of California, Irvine). The M1 receptor inhibitory activity was determined by Fluorometric Imaging Plate Reader (FLIPR). The assay was performed as reported earlier with slight modification [29]. Briefly, the cells were seeded into black wall, clear-bottom 96-well plates at a density of 60,000 cells per well. Twenty-four hours later the medium was removed and replaced with 100 µl of dye loading solution (2 µM Fluo-4 AM dissolved in FLIPR buffer, which consists of pluronic acid in 1 × Hank's buffer supplemented with 20 mM HEPES, pH 7.4) for 1 h at 37 °C. The cells were then washed 3 times with FLIPR buffer prior to FLIPR assay. The compounds were dissolved in dimethyl sulphoxide (DMSO) and diluted with FLIPR buffer. For antagonist tests, the compounds were first incubated with the cell for 10 min, before the addition of 50 nM carbachol to induce the activity of M1 receptor. Data were expressed as fluorescence (arbitrary units) versus time.

2.4. Data processing

IC₅₀ values and curve fitting were determined using Graphpad Prism (GraphPad Software, Inc., San Diego, CA). Data from each dose–response curve were normalized to the carbachol (50 nM) stimulation. At least three independent experiments were performed. Data are presented as means ± standard error of the mean (SEM).

Fig. 1. Structures of compounds 1–11 isolated from *S. tangutica*. Seven HCA amides (1–7): scotamine A–D (1–4), N¹, N¹⁰-di-dihydrocaffeoylspermidine (5), caffeoylputrescine (6), N¹-caffeoyl-N³-dihydrocaffeoylspermidine (7); one cinnamic acid amide: carboxyscotangamine A (8) and three well-known anticholinergic compounds (9–11): scopolamine (9), atropine (10) and anisodine (11).

3. Results and discussion

Eleven alkaloids have been isolated from *S. tangutica* [11,12]. They include seven HCA amides (1–7): scotamine A–D (1–4), N¹, N¹⁰-di-dihydrocaffeoylspermidine (5), cafeoylputrescine (6), N¹-caffeoyl-N³-dihydrocaffeoylspermidine (7), one cinnamic acid amide: carboxyscotangamine A (8) and three well-known anticholinergic compounds (9–10): scopolamine (9), atropine (10) and anisodine (11). Their structures were shown in Fig. 1.

These alkaloids as well as the *S. tangutica* crude extract, from where they were isolated, were tested for their abilities at inhibiting the muscarinic M1 receptor. As shown in Fig. 2A, the crude extract was able to dose-dependently inhibit the carbacol-induced calcium mobilization. At a concentration of 2 µg/mL the M1 receptor activation is practically totally inhibited.

Then the purified alkaloids were tested for their abilities to antagonize the M1 receptor. First the well-known anticholinergic compounds scopolamine (9), atropine (10) and anisodine (11) displayed potent inhibitory activity against M1 receptor (Fig. 2B) with IC₅₀ values of 1.6, 28 and 82 nM, respectively. These values are in agreement with published data [4,8]. These compounds could serve as positive controls in the assay.

We then accessed whether these seven HCA amides and one cinnamic acid amide have inhibitory effects on M1 receptors. We screened the activities of these eight compounds at a concentration of 10 µM in CHO-M1 cells. As shown in Fig. 2C, compounds 1 and 6 were able to inhibit carbacol induced M1 receptor activities by 35–40% and potent inhibition was noted with compounds 2–5 and 7 at a range of 80–100%.

The concentration–inhibition relationships for compounds 2–5 and 7 were further studied (Fig. 2D). scotamine D (4) displayed the best inhibitory activity against M1 receptor with an IC₅₀ value of 32 nM, followed by scotamine B (compound 2, 397 nM), N¹-caffeoyl-N³-dihydrocaffeoylspermidine (compound 7, 863 nM), N¹⁰-di-dihydrocaffeoylspermidine (compound 5, 2.9 µM) and scotamine C (compound 3, 7.8 µM). To study the selectivity of the response, these compounds were tested in HEK293T cells expressing ORL-1 receptor and displayed no activities (data not shown). Here compared to compound 9–11, compound 1–4 and 8 are newly discovered natural products, therefore our findings provide their first biological assessment.

Because the conjugated molecules 2–5 contain caffeic acid and polyamine backbones, we tested whether caffeic acid itself exhibits activity at the M1 receptor. However, caffeic acid did not show any activity against M1 receptor (Fig. 2C) at concentrations up to 10 µM. Regarding the polyamine backbone, we found that the short chain amine conjugates (compounds 1, 6 and 8) are much less active than the longer chain ones (compounds 2–5 and 7). This points at the importance of the polyamine chain in the binding to the M1 receptor. The polyamine backbone has been proposed as a key pharmaceutical skeleton in the binding of different receptors [19]. Methoctramine is the prototype polymethylene tetraamine for antagonism of mAChRs [30]. A more complete analysis of the role of the polyamine chemical structure on biological activity will require a higher number of compounds and remains to be studied.

4. Conclusions

Our study shows that HCA amides can inhibit the M1 receptor activity as well as the crude *S. tangutica* extracts. Therefore these HCA

Fig. 2. The antagonist effects of the extracts and compounds from *S. tangutica*. (A) The dose-dependent inhibitory effects of the crude extract against M1 receptor; (B) dose–response curves of compounds 9–11 for inhibition of carbacol-induced Ca²⁺ mobilization in CHO-M1 cells; (C) screening of compounds 1–8 and caffeic acid (CA) in CHO-M1 cells at a concentration of 10 µM; (D) dose–response curves of compounds 2–5 and 7 for inhibition of carbacol-induced Ca²⁺ mobilization in CHO-M1 cells. The data represented three separate experiments. Data are mean ± SEM.

amides may participate in the therapeutic action of *S. tangutica* extracts. Our study revealed that not only anisodine and scopolamine, but also the presence of some of HCA amides are responsible for the anticholinergic effects of this plant medicine. Our findings thus provided additional molecular basis for the traditional use of *S. tangutica* against spasm. Noteworthy, mAChR antagonists were also among the first drug for Parkinson's disease (PD) and still are widely used today [31,32]. Thus, these HCA amides described here may also have a therapeutic potential in PD. Further studies will clarify the selectivity of these compounds at different subtypes of mAChRs.

Acknowledgments

This work was supported by National Science Foundation of China (81402806) and National Institute of Health Grants (MH60231, AT00801).

References

- [1] G.Q.E. Wang, Collection of Chinese Herbal Medicines, 3th ed. People's Medical Publishing House, Beijing, 2014.
- [2] eFloras (2008), Published on the Internet <http://www.efloras.org> [accessed 1 September 2015] Missouri Botanical Garden, St. Louis, MO & Harvard University Herbaria, Cambridge, MA.
- [3] D.R. Varma, T.L. Yue, Adrenoceptor blocking properties of atropine-like agents anisodamine and anisodine on brain and cardiovascular tissues of rats, *Br. J. Pharmacol.* 87 (1986) 587–594.
- [4] W.W. Zhang, M.K. Song, Y.Y. Cui, H. Wang, L. Zhu, Y.Y. Niu, et al., Differential neuropsychopharmacological influences of naturally occurring tropane alkaloids anisodamine versus scopolamine, *Neurosci. Lett.* 443 (2008) 241–245.
- [5] R.J. Xiu, D.E. Hammerschmidt, P.A. Coppo, H.S. Jacob, Anisodamine inhibits thromboxane synthesis, granulocyte aggregation, and platelet aggregation. A possible mechanism for its efficacy in bacteremic shock, *JAMA* 247 (1982) 1458–1460.
- [6] R.E. Burke, The relative selectivity of anticholinergic drugs for the M1 and M2 muscarinic receptor subtypes, *Mov. Disord.* 1 (1986) 135–144.
- [7] H.P. Rang, Pharmacology, Churchill Livingstone, Edinburgh, 2003.
- [8] F. Huang, P. Buchwald, C.E. Browne, H.H. Farag, W.M. Wu, F. Ji, et al., Receptor binding studies of soft anticholinergic agents, *AAPS PharmSci.* 3 (2001), E30.
- [9] M.P. Caulfield, Muscarinic receptors—characterization, coupling and function, *Pharmacol. Ther.* 58 (1993) 61.
- [10] M.E. Richard, C. A., P.D. Michael, H. Sharath, Muscarinic receptor ligands and their therapeutic potential, *Curr. Opin. Chem. Biol.* 3 (1999) 7.
- [11] Z. Long, Z. Guo, X. Xue, X. Zhang, X. Liang, Two-dimensional strong cation exchange/positively charged reversed-phase liquid chromatography for alkaloid analysis and purification, *J. Sep. Sci.* 36 (2013) 3845–3852.
- [12] Z. Long, Y. Zhang, Z. Guo, L. Wang, X. Xue, X. Zhang, et al., Amide alkaloids from *Scopolia tangutica*, *Planta Med.* 80 (2014) 1124–1130.
- [13] J. Luo, C. Fuell, A. Parr, L. Hill, P. Bailey, K. Elliott, et al., A novel polyamine acyltransferase responsible for the accumulation of spermidine conjugates in *Arabidopsis* seed, *Plant Cell* 21 (2009) 318–333.
- [14] C. Fellenberg, C. Bottcher, T. Vogt, Phenylpropanoid polyamine conjugate biosynthesis in *Arabidopsis thaliana* flower buds, *Phytochemistry* 70 (2009) 1392–1400.
- [15] J. Martin-Tanguy, F. Cabanne, E. Perdrizet, C. Martin, The distribution of hydroxycinnamic acid amides in flowering plants, *Phytochemistry* 17 (1978) 1927–1928.
- [16] M. Nimtz, M. Bokern, B. Meurer-Grimes, Minor hydroxycinnamic acid spermidines from pollen of *Quercus dentata*, *Phytochemistry* 43 (1995) 487–489.
- [17] V.S. Sobolev, A.A. Sy, J.B. Gloer, Spermidine and flavonoid conjugates from peanut (*Arachis hypogaea*) flowers, *J. Agric. Food Chem.* 56 (2008) 2960–2969.
- [18] M. Bokern, L. Witte, V. Wray, M. Nimtz, B. Meurer-Grimes, Trisubstituted hydroxycinnamic acid spermidines from *Quercus dentata* pollen, *Phytochemistry* 39 (1995) 1371–1375.
- [19] C. Melchiorre, M.L. Bolognesi, A. Minarini, M. Rosini, V. Tumiatti, Polyamines in drug discovery: from the universal template approach to the multitarget-directed ligand design strategy, *J. Med. Chem.* 53 (2010) 5906–5914.
- [20] Y. Li, K. Popaj, M. Lochner, H. Geneste, R. Budriesi, A. Chiarini, et al., Analogues of polyamine alkaloids and their synthetic advantages, *Farmacol.* 56 (2001) 127–131.
- [21] S. Fixon-Owo, F. Levasseur, K. Williams, T.N. Sabado, M. Lowe, M. Klose, et al., Preparation and biological assessment of hydroxycinnamic acid amides of polyamines, *Phytochemistry* 63 (2003) 315–334.
- [22] K.D. McCormick, J. Meinwald, Neurotoxic acylpolyamines from spider venoms, *J. Chem. Ecol.* 19 (1993) 2411–2451.
- [23] I.S. Blagbrough, E. Moya, S. Taylor, Polyamines and polyamine amides from wasps and spiders, *Biochem. Soc. Trans.* 22 (1994) 888–893.
- [24] K. Stromgaard, L.S. Jensen, S.B. Vogensen, Polyamine toxins: development of selective ligands for ionotropic receptors, *Toxicol.* 45 (2005) 249–254.
- [25] G. Estrada, E. Villegas, G. Corzo, Spider venoms: a rich source of acylpolyamines and peptides as new leads for CNS drugs, *Nat. Prod. Rep.* 24 (2007) 145–161.
- [26] T.I. Bonner, N.J. Buckley, A.C. Young, M.R. Brann, Identification of a family of muscarinic acetylcholine receptor genes, *Science* 237 (1987) 527–532.
- [27] M.P. Caulfield, N.J. Birdsall, International union of pharmacology. XVII. Classification of muscarinic acetylcholine receptors, *Pharmacol. Rev.* 50 (1998) 279–290.
- [28] Z. Long, C. Wang, Z. Guo, X. Zhang, L. Nordahl, J. Zeng, et al., A non-aqueous solid phase extraction method for alkaloid enrichment and its application in the determination of hyoscyamine and scopolamine, *Analyst* 137 (2012) 1451–1457.
- [29] Y. Zhang, J. Xu, Z. Wang, X. Zhang, X. Liang, O. Civelli, BmK-YA, an enkephalin-like peptide in scorpion venom, *PLoS One* 7 (2012), e40417.
- [30] M. Rosini, R. Budriesi, M.G. Bixel, M.L. Bolognesi, A. Chiarini, F. Hucho, et al., Design, synthesis, and biological evaluation of symmetrically and unsymmetrically substituted methoctramine-related polyamines as muscarinic nicotinic receptor non-competitive antagonists, *J. Med. Chem.* 42 (1999) 5212–5223.
- [31] J.J. Chen, D.M. Swope, Pharmacotherapy for Parkinson's disease, *Pharmacotherapy* 27 (2007) 161S–173S.
- [32] R. Katzenschlager, C. Sampaio, J. Costa, A. Lees, Anticholinergics for symptomatic management of Parkinson's disease, *Cochrane Database Syst. Rev.* (2003), CD003735.