Biochemical Pharmacology 79 (2010) 1815–1826

Contents lists available at ScienceDirect

Biochemical Pharmacology

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

Falcarinol is a covalent cannabinoid CB_1 receptor antagonist and induces pro-allergic effects in skin

Marco Leonti [a], Laura Casu [a], Stefan Raduner [b], Filippo Cottiglia [a], Costantino Floris [c], Karl-Heinz Altmann [b], Jürg Gertsch [b,*]

[a] Dipartimento Farmaco Chimico Tecnologico, Università di Cagliari, Facoltà di Farmacia, Via Ospedale 72, 09124 Cagliari, Italy
[b] Institute of Pharmaceutical Sciences, ETH Zürich, Wolfgang-Pauli-Strasse 10, CH-8057 Zurich, Switzerland
[c] Dipartimento di Scienze Chimiche, Università di Cagliari, Complesso di Monserrato, 09042 Monserrato, Italy

ARTICLE INFO

Article history:
Received 7 December 2009
Accepted 24 February 2010

Keywords:
Falcarinol
Skin irritation
CB1 cannabinoid receptor
Anandamide
Endocannabinoid system

ABSTRACT

The skin irritant polyyne falcarinol (panaxynol, carotatoxin) is found in carrots, parsley, celery, and in the medicinal plant *Panax ginseng*. In our ongoing search for new cannabinoid (CB) receptor ligands we have isolated falcarinol from the endemic Sardinian plant *Seseli praecox*.

We show that falcarinol exhibits binding affinity to both human CB receptors but selectively alkylates the anandamide binding site in the CB_1 receptor (K_i = 594 nM), acting as covalent inverse agonist in CB_1 receptor-transfected CHO cells. Given the inherent instability of purified falcarinol we repeatedly isolated this compound for biological characterization and one new polyyne was characterized. In human HaCaT keratinocytes falcarinol increased the expression of the pro-allergic chemokines IL-8 and CCL2/MCP-1 in a CB_1 receptor-dependent manner. Moreover, falcarinol inhibited the effects of anandamide on TNF-alpha stimulated keratinocytes. *In vivo*, falcarinol strongly aggravated histamine-induced oedema reactions in skin prick tests. Both effects were also obtained with the CB_1 receptor inverse agonist rimonabant, thus indicating the potential role of the CB_1 receptor in skin immunopharmacology. Our data suggest anti-allergic effects of anandamide and that falcarinol-associated dermatitis is due to antagonism of the CB_1 receptor in keratinocytes, leading to increased chemokine expression and aggravation of histamine action.

1. Introduction

The endocannabinoid system (ECS) comprises the G-protein coupled cannabinoid receptors (CB_1 and CB_2) and potentially also the orphan receptor GPR55, several endogenous arachidonic acid-derived ligands, their regulatory transport systems, and their hydrolytic enzymes (for reviews see [1–3]). Although the CB_1 receptor is the predominant cannabinoid receptor expressed in the brain it is also present in numerous peripheral tissues, often in concert with the peripheral CB_2 receptor. The major endocannabinoids arachidonoyl ethanolamide (anandamide) (Fig. 1) and 2-arachidonoyl glycerol (2-AG) (Fig. 1) non-selectively and to different degrees activate both CB receptors at nM concentrations. In skin, the activation of the ECS mediates a rather complex physiology, which remains to be investigated (for a recent review see [4]). While the CB_2 receptor has been shown to potentially transduce pro-inflammatory effects via 2-AG [5], an overall increased endocannabinoid tone appears to be beneficial [6]. Studying CB knockout mice, Karsak et al. have observed that the ECS and the CB_1 receptor can inhibit the pathogenesis of allergic contact dermatitis [6]. Interestingly, in their study it was shown that selective activation of the CB_2 receptor in skin worsened the allergic reaction, thus confirming previous data obtained by Oka et al. [7], while blockage of the CB_2 receptor by the inverse agonist SR144528 paradoxically led to a similar result [6]. By contrast, CB_1 agonists such as Δ^9-tetrahydrocannabinol (THC) and inhibition of anandamide degradation are considered to be promising therapeutic strategies to treat different forms of dermatitis [4,6]. Overall, anandamide acting via both CB_1 and CB_2 receptors appears to mediate skin protective effects. While an immunological role of the ECS in skin has already been shown, and both CB_1 and CB_2 receptors and endocannabinoids have been detected in keratinocytes and fibroblasts in the epidermis [8,9], the pleiotropic endocannabinoid signals involved in skin inflammation are complex and remain poorly understood. In this study we have identified the fatty acid-derived natural product falcarinol which possesses a reactive polyyne structure, as new functional CB receptor ligand. Interestingly, falcarinol has previously been reported to exert pharmacological effects,

* Corresponding author at: Institute of Biochemistry and Molecular Medicine, University of Bern, Bühlstrasse 28, CH-3012 Bern, Switzerland.
E-mail address: gertsch@mci.unibe.ch (J. Gertsch).

0006-2952/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2010.02.015

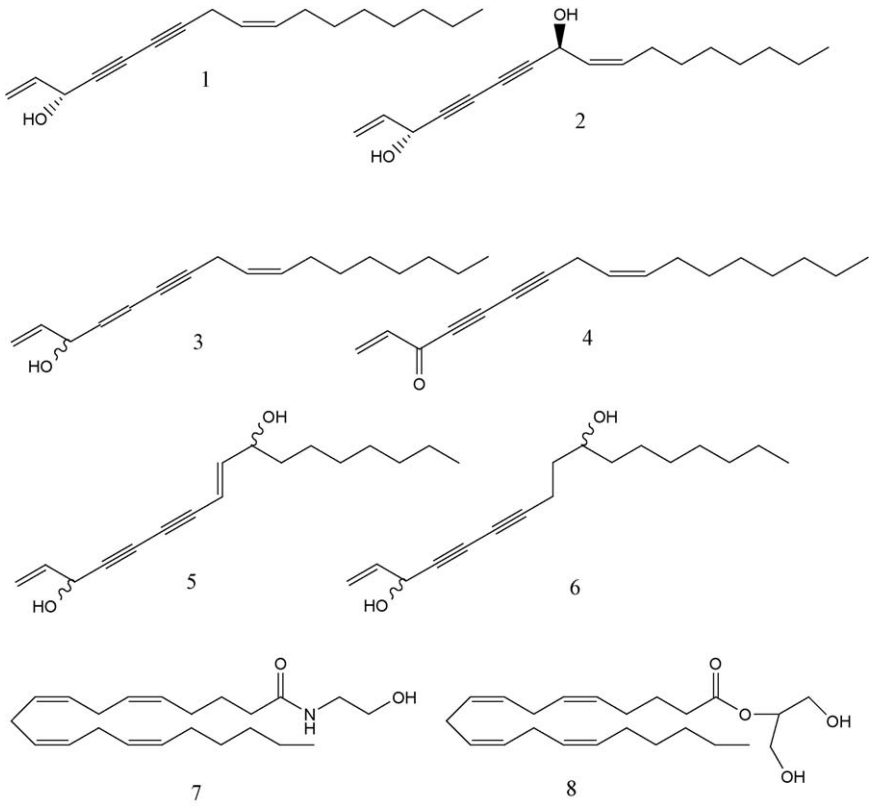

Fig. 1. Structures of natural polyynes and decomposition products (**1**–**6**), and endocannabinoids (**7**;**8**). (*R*)-Falcarinol ((*Z*)-heptadeca-1,9-diene-4,6-diyn-3-ol) (**1**), 3(*R*),8(*S*)-falcarindiol ((*Z*)-heptadeca-1,9-diene-4,6-diyn-3,8-diol) (**2**); (4*E*,9*Z*)-1,4,9-heptadecatriene-6-yn-3-ol or 4,5-dihydrofalcarinol (4,5-dihydropanaxynol) or ginsenoyne J (**3**); falcarinone ((*Z*)-heptadeca-1,9-diene-4,6-diyn-3-one) (**4**); *E*-heptadeca-1,8-diene-4,6-diyne-3,10-diol (**5**); dihydroseselidiol (heptadeca-1-ene-4,6-diyne-3,10-diol) (**6**); *N*-arachidonoyl ethanolamine (anandamide) (**7**); 2-arachidonoyl glycerol (2-AG) (**8**).

including skin irritation (dermatitis) and potential anti-carcinogenic effects in colon [10–13]. However, to date no protein targets for falcarinol have been identified. Here we show that falcarinol non-selectively binds to both CB receptors, but selectively alkylates the CB_1 receptor and induces functional signals at the CB_1 receptor. In keratinocytes these signals may lead to up-regulation of pro-inflammatory chemokines. Our data further suggest that this interaction is similar to the molecular interaction of the CB_1 receptor-selective inverse agonist rimonabant (SR141716) which is known to potentially mediate pro-inflammatory effects in skin [6]. To our knowledge, this is the first report on skin inflammation induced by externally applied CB_1 receptor antagonists (inverse agonists).

2. Materials and methods

2.1. Plant material

Stems of the endemic *Seseli praecox* (Gamisans) Gamisans (Apiaceae), a chamaephyte species growing on calcareous substrate were collected near Baunei (Ogliastra, Sardinia) in October 2006.

2.2. Chemicals

[^{3}H]-8-OH-DPAT (1 mCi/ml, 106–170 Ci/mmol), [^{3}H]-GR65630 (1 mCi/ml, 77.2 Ci/mmol), [^{3}H]-CP55,940 (1 mCi/ml, 126 Ci/mmol), [^{3}H]-RTX (1 mCi/ml, 43 Ci/mmol), [^{3}H]FMLP, and their respective ^{3}H unlabelled analogs, as well as the Ultima Gold scintillation cocktail were obtained from PerkinElmer, Switzerland. [^{3}H]Anandamide (223 Ci/mmol) was purchased from NEN Life Science Products. Rimonabant (SR141716) and SR144528 were obtained as a kind gift from Sanofi Synthelabo, France. Anandamide and WIN55,212-2 were purchased from Tocris, UK. Histamine hydrochloride was obtained from Sigma Aldrich, Switzerland. Silica gel (40–63 μm) and TLC plates with silica gel 60 and fluorescence indicator 254 nm was obtained from Merck, Solvents were obtained from VWR International, Germany. All other chemicals were from Fluka Chemie AG, Switzerland.

2.3. Receptor screen

Human CB_1, CB_2, 5-HT_{1A}, 5-HT_3, FPRL-1 overexpressing HEK-293 membranes were obtained from PerkinElmer, Switzerland. CHO-K1 cells stably expressing human TRPV1 were a gift from Dr. Zoltan Sandor, University of Debrecen, Hungary. Membrane preparations (between 7 and 20 μg) were resuspended in 0.2 ml (final volume) of binding buffer (50 mM Tris–HCl, 2.5 mM EGTA, 5 mM $MgCl_2$, 0.5 mg/ml fatty acid free BSA, pH 7.4). Receptor concentrations (B_{max}) were 1.7–3.2 pmol/mg of protein. The nonspecific binding of the radioligand was determined in the presence of excess ligands. Radioligand saturated membrane preparations were incubated with 10 μM of falcarinol. After 90 min of incubation, the suspension was rapidly filtered through 0.05% polyethyleneimine presoaked GF/C glass fiber filters on a 96-well cell harvester and washed nine times with 0.5 ml of ice-cold washing buffer (50 mM Tris–HCl, 2.5 mM EGTA, 5 mM MgCl2, 2% BSA, pH 7.4). Radioactivity on filters was measured with a Beckman LS 6500. A displacement of more than 50% of the radioligands by 10 μM of competitor was considered to be significant. Using this setup, the nonlabeled analogs of the respective radioligands displaced the radioligands by more than 70%.