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Homologues and Isomers of Noladin Ether, a Putative Novel Endocannabinoid: Interaction with Rat Cannabinoid CB_1 Receptors

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Abstract—Two regioisomers and 13 analogues of the putative endocannabinoid noladin ether (2-arachidonyl glyceryl ether, 2-AGE, **1**) were synthesized and tested for their interaction with CB_1 receptors in rat brain membranes. The results showed that a C-20 tetra-unsaturated moiety is necessary for high affinity, and that a series of alkyl glyceryl ethers of potential occurrence in brain tissues have less affinity than 2-AGE for CB_1 receptors.

Noladin ether (**1**),[1] the 2-glyceryl ether of arachidonyl alcohol, is the latest polyunsaturated fatty acid derivative to be proposed as an endogenous ligand for the cannabinoid CB_1 receptor,[2] one of the two recognition sites for marijuana's psychoactive principle (−)-Δ^9-tetrahydrocannabinol (THC) and for the two previously described endocannabinoids,[3] *N*-arachidonoyl-ethanolamine (anandamide, AEA, **2**) and 2-arachidonoyl glycerol (2-AG, **3**). While the chemical bases for the interaction of AEA and 2-AG with cannabinoid CB_1 receptors have been investigated in detail,[4] nothing is known on the structure–activity relationships underlying the cannabimimetic activity of alkyl glyceryl ethers. Hence, two enantiomeric 2-AGE regioisomers (*sn*-1 and *sn*-3 arachidonyl glyceryl ether) and a series of 2-AGE analogues (Table 1) were prepared and tested for CB_1 affinity in specific binding assays (Fig. 1).

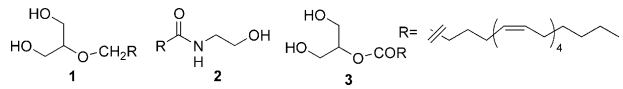

Figure 1. Chemical structures of endocannabinoids.

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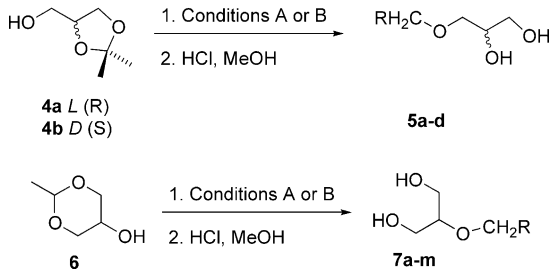
Results and Discussion

The terminal glyceryl ethers **5a–d** were obtained by reacting L- or D-solketal (glycerol acetonide, **4a**,**b**) with arachidonyl mesylate, affording, after deprotection, the *sn*-1- and *sn*-3 ethers **5a**,**b**, respectively (Scheme 1). The 2-alkyl glyceryl ethers **7a–m** were obtained by treatment of 1,3-benzilydenglycerol (**6**)[1] with fatty alcohol mesylates (final compounds **7a–i**) or isoprenoid bromides (final compounds **7l**,**m**), followed by acidic deprotection (HCl in MeOH) (Scheme 1). The base employed in the etherification was dimsyl anion (Scheme 1, Conditions B), except when skipped double bonds were present. In this case, KOH in toluene had to be employed (Scheme 1, Conditions A).[1]



Scheme 1. Synthesis of the glyceryl ethers **5a–d** and **7a–m**. Conditions A: RCH_2–OMs, KOH, benzene, 40 °C; Conditions B: RCH_2–OMs (RCH_2–Br for the isoprenoid residues), NaH, DMSO, tetrabutylammonium iodide, rt. For the nature of R and the yields, see Table 1.

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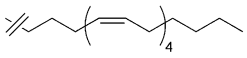
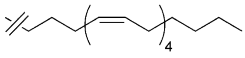
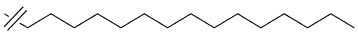
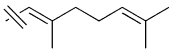
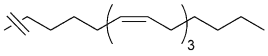
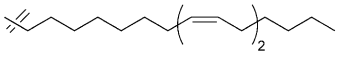
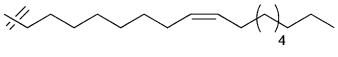
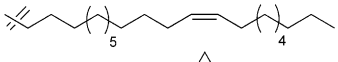
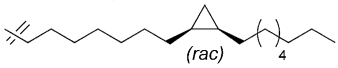
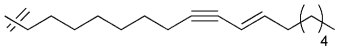
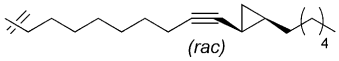
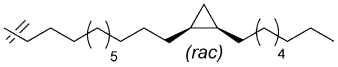
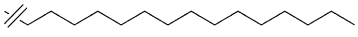
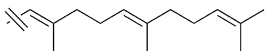
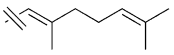
Table 1. Affinity for rat cannabinoid CB_1 receptors of noladin ether analogues[a,b]

Entry	R	Etherification site (conditions, yield)	K_i (μM)	K_{rel}
5a		*sn*-1 (A, 34%)	5.0±0.8	1250
5b		*sn*-3 (A, 33%)	2.5±0.4	625
5c		*sn*-3 (B, 48%)	> 50	> 12,500
5d		*sn*-1 (B, 52%)	> 50	> 12,500
7a		2 (A, 21%)	13.9±1.2	3475
7b		2 (A, 26%)	22.5±1.8	5625
7c		2 (B, 38%)	25.6±2.1	6400
7d		2 (B, 39%)	> 50*	> 12,500
7e		2 (B, 47%)	> 50	> 12,500
7f		2 (B, 26%)	> 50*	> 12,500
7g		2 (B, 36%)	> 50	> 12,500
7h		2 (B, 41%)	> 50	> 12,500
7i		2 (B, 64%)	> 50	> 12,500
7l		2 (B, 50%)	> 50	> 12,500
7m		2 (B, 77%)	> 50	> 12,500

[a]The site of etherification, stereochemistry and type of chain are shown (see Scheme 1).
[b]The affinity is expressed in terms of absolute K_i (μM) for the displacement of [^{3}H]SR141716A from cannabinoid CB_1 receptors in rat brain membrane preparations, as well as in terms of relative K_i, that is $K_{rel} = K_i/K_i$ of HU-210. The K_i for the displacement by HU-210 of [^{3}H]SR141716A from rat brain membranes was 4.0±0.3 nM. For 2-AGE, $K_i = 2.5 \pm 0.3$ μM; $K_{rel} = 625$. The symbol * indicates that it was possible to measure some displacement at a 50 μM concentration.

This change gave overall lower yields, but avoided double bond conjugation and preserved homoconjugation.[5]

The choice of the alkyl moiety, though far from exhaustive, was done making reference to fatty and isoprenoid alcohols of potential natural occurrence as glyceryl ethers. Since arachidonic acid is not easily amenable to point mutations, modification of the olefin moiety (cyclopropanation, isomerization and inclusion in a conjugate system) were carried out for the C-18 monounsaturated alkyl residue. The fifteen alkyl glyceryl ethers prepared were then tested as cannabinoid CB_1 receptor ligands using a widely accepted procedure,[2,6] namely the equilibrium competition of increasing concentrations of the test compounds with a fixed concentration of the high affinity CB_1 ligand, [^{3}H]SR141716A, in rat brain membranes, which contain high levels of CB_1 receptors.[2,7]

The K_i value for the compounds tested are reported in Table 1. The binding affinity we measured for 2-AGE ($K_i = 2.5$ μM) in our assay system was about 100-fold higher than that reported in synaptosomal membranes from rat brain (without brainstem) using [^{3}H]HU-243 as radioligand, and centrifugation instead of filtration to separate bound from unbound ligand.[1] Variations of this type are not uncommon.[2,7] On the other hand, the comparison of the ratios between the K_i constants of 2-AGE and HU-210 measured in rat brain membranes with [^{3}H]SR141716A and in rat brain synaptosomes with [^{3}H]HU-243 showed very similar values, with 2-AGE being about 2.5 orders of magnitude less potent than HU-210 as a CB_1 receptor ligand in both cases.[8] Therefore, the relative CB_1 affinity constants within a series of homologues should always be the same, independently of the binding assay used. For this reason, Table 1 shows not only the K_i values of each compounds but also their ratios with the K_i value calculated here for HU-210 (K_{rel}).

The first finding of this study is that the two enantiomeric 2-AGE regioisomers *sn*-1- and *sn*-3-arachidonyl glyceryl ethers (**5a**,**b**) exhibit affinity for CB_1 receptors similar to that of 2-AGE (Fig. 2). However, the enan-

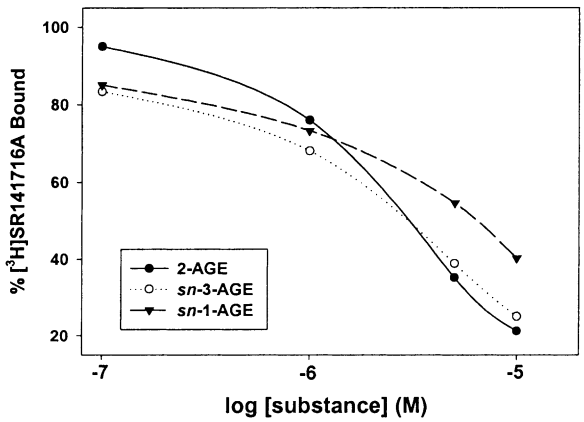


Figure 2. Displacement by increasing doses of noladin ether (2-AGE) or its positional isomers, *sn*-1 and *sn*-3-arachidonyl glyceryl ethers, of [^{3}H]SR141716A from cannabinoid CB_1 receptors in rat brain membrane preparations. The displacement assay was carried out as described in the Methods. Data points are means of three separate experiments. Error bars are not shown for the sake of clarity and were never higher than 5%.

tiomer more likely to be naturally occurring (*sn*-1, **5a**) is 2-fold less potent than 2-AGE (**1**) and its *sn*-3 enantiomer **5b** (Table 1). This is not surprising, since a high degree of enantioselectivity for CB_1 binding was reported for several chiral cannabinoid ligands, including THC itself.[2,7] Our finding is important in view of the controversy generated by the discovery of 2-AGE in animal tissues.[1] Before the discovery of 2-AGE, the only natural glyceryl ethers known were those with the alkyl (or alkenyl) chain in the *sn*-1 position, as exemplified by PAF and the plasmalogens.[9] Our findings show that, even if the *sn*-1 regioisomer (**5a**) of 2-AGE was to be recognized as the most abundant naturally occurring arachidonyl glyceryl ether in mammalian brain, this compound would have lower affinity for CB_1 receptors than 2-AGE.

The other original finding of this study is that, as with AEA and 2-AG,[10] also for alkyl gliceryl ethers a polyunsaturated C-20 chain is important for cannabinoid activity. Thus, shortening of the side chain without a substantial decrease of the overall degree of unsaturation in terms of double bonds per carbon atoms, as featured in the C-18 ω-6 polyunsaturated 2-AGE analogue 2-γ-linolenoyl-glyceryl ether (**7a**), caused a 5.5-fold loss of potency (Table 1). Furthermore, within the C-18 series of analogues, affinity for CB_1 receptors gradually decreased with saturation, the 2-linoleyl (**7b**) and 2-oleyl (**7c**) homologues being 9- and 10-fold less potent than 2-AGE, and 1.6- and 1.85-fold less potent than 2-γ-linolenoyl-glyceryl ether (**7a**), respectively (Table 1). In compounds from the C-18 series, one *cis* double bond was sufficient to impart a certain activity at CB_1 receptors, but this did not hold true for higher homologues, since the C-22 homologue, 2-erucyl glyceryl ether (**7d**), was almost inactive. Cyclopropanation of 2-oleyl-glyceryl ether as in **7e**, or replacement of the double bond with a *trans*-enine construct as in **7f** were both detrimental for activity, and the residual affinity of 2-ximenyl-glyceryl ether (**7f**) was totally abolished by cyclopropanation of the double bond (**7g**). Likewise, the methylene derivative of 2-erucyl glyceryl ether (**7h**) was devoid of significant activity. Finally, replacement of the arachidonyl moiety with three widespread alkyl groups (palmityl (**7i**), farnesyl (**7l**) and geranyl (**7m**)) also abolished activity. No activity was also observed for the *sn*-3 palmityl and *sn*-1 geranyl ethers (**5c** and **5d**, respectively) (Table 1).

Taken together, our findings: (1) extend to 2-AGE previous observations on the relevance of a tetra-unsaturated C-20 chain for high affinity at CB_1 receptors;[4,10] and (2) indicate that a range of alkyl glyceryl ethers of potential natural occurrence would not be more potent endocannabinoids than 2-AGE. These results support the view that 2-AGE, rather than its homologues and analogues, is an endocannabinoid, and provide information on the structural basis of its interactions with the CB_1 receptor binding site. The hydrophilic head of 2-AGE and its regioisomers provide ample opportunity for chemical modification. Given the increased potency attained by modifications of the polar moiety of other endocannabinoids and the metabolic stability of glyceryl ethers, 2-AGE analogues that retain the arachidonyl moiety and bear suitable modifications in the glyceryl head might represent an interesting opportunity to overcome the pharmacokinetics shortcomings of other types of endocannabinoids.

Acknowledgements

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5. All compounds were characterized by IR, ^{1}H and ^{13}C NMR, as well as MS data. Reaction conditions A were adopted without modification from ref 1. The synthesis of 2-farnesyl gyceryl ether (**7l**) is presented as an example of procedure B: To a suspension of NaH (60% dispersion in mineral oil, 70 mg, 1.63 mMol, 1.5 mol equiv) in dry THF (5 mL), 1,3-

benzylidenglycerol (196 mg, 1.09 mMol, 1 mol. equiv) was added. When gas evolution ceased, tetrabutylammonium iodide (20 mg, cat.) and *E,E*-farnesylbromide (310 mg, 295 μL, 1.09 mMol) were added. After stirring at rt for 3 h, the reaction was worked up by dilution with petroleum ether–EtOAc 3:1 and washed with NH_4Cl. After washing with brine, drying (Na_2SO_4), and evaporation, the residue was dissolved in MeOH (5 mL) and treated with 500 μL of a soln obtained by reacting AcCl (560 μL) in MeOH (10 mL). After 2 h, the reaction was worked up by neutralization with $NaHCO_3$ and extraction with EtOAc. After removal of the solvent, the residue was purified by CC (13 g silica gel, petroleum ether–EtOAc 9:1 as eluant) to give 161 mg (50%) **7l** as a colourless oil.

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8. In separate experiments, we observed that the K_i for 2-AGE using [^{3}H]HU-243 was 0.70 ± 0.16 μM ($n = 3$) with rat brain membranes (Di Marzo, V.; Bisogno, T.; Ligresti, A. unpublished observations).
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