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ARACHIDONIC ACID AMIDE INHIBITORS OF GAP JUNCTION CELL–CELL COMMUNICATION

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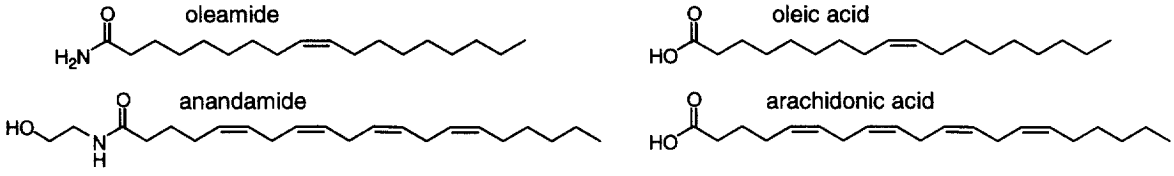
Abstract. A series of arachidonic acid amides including anandamide and arachidonamide that act as potent inhibitors of the rat glial cell gap junction is described.

Oleamide,[1,2] the prototypical member[1–4] of a growing class of endogenous fatty acid primary amides,[5] was found to accumulate in the cerebrospinal fluid under conditions of sleep deprivation[2,6,7] and induce physiological sleep in animals.[1,2] In a structurally specific manner, oleamide modulates the serotonergic system,[8–10] benzodiazepine-sensitive $GABA_A$ receptors,[11] blocks glial gap junction cell–cell communication,[12,13] and exhibits the characteristic in vivo analgesic and cannabinoid effects of anandamide in mice[14] albeit without cannabinoid receptor binding.

Anandamide[3,4] binds to both the central CB1 and peripheral CB2 cannabinoid receptors[3,4] through which it is thought to exhibit its analgesic and cannabinoid effects, blocks glial gap junction cell–cell communication,[12,13,15] differentially modulates the serotonergic system,[10,16] and exhibits a number of additional in vitro and in vivo biological properties[3] including the modulation of sleep and memory.[17]



Oleamide was found to inhibit the gap junction-mediated chemical and electrical transmission in rat glial cells, but had no inhibitory effect on mechanically-stimulated or glutamate-induced calcium wave propagation thereby decoupling two previously indistinguishable glial cell communication pathways.[12] An examination of the primary amides of a complete set of natural and synthetic fatty acids established that the effective inhibitors of gap junction-mediated chemical and electrical transmission in rat glial cells fall into two classes of which oleamide and arachidonamide were the prototypical members of the two classes.[13] Of these, oleamide was the more potent and

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the structural requirements for inhibition of the gap junction by this class of monounsaturated fatty acid amides were found to be well defined. It required a chain length of 16–24 carbons of which 16–18 was optimal, a terminal polarized carbonyl group capable of accepting but not necessarily donating a hydrogen bond, a hydrophobic methyl terminus, and a well placed *cis* double bond.[13]

Of special note was the effective gap junction inhibitory properties of a structurally well-defined series of secondary and tertiary amides of oleic acid. These studies, which included the examination of oleyl ethanolamide and arachidonyl ethanolamide (ananamide), indicated that the corresponding primary amides were more potent. Moreover, oleyl ethanolamide was not distinguishable from a range of simple secondary and tertiary amides suggesting there is nothing unique to the behavior of the ethanolamides. In fact, not only did the hydroxyl group of oleyl ethanolamide not contribute to its gap junction inhibitory properties, but it was found to potentially diminish them. Given the biological importance of anandamide and the identification of the arachidonamide class of gap junction inhibitors, herein we detail the examination of the effect of amide structural features on the gap junction inhibitory properties of arachidonamides.

Synthesis. The arachidonamides shown in Table 1 were prepared in a single operation from arachidonic acid by first conversion to the acid chloride (1.2 equiv $(COCl)_2$, cat. DMF, CH_2Cl_2, 25 °C, 1–3 h) and subsequent treatment with the appropriate amine (1–2 equiv, CH_2Cl_2, 25 °C, 1–4 h, 80–100%).

Gap Junction Inhibition. The inhibition of the rat glial cell gap junction was assessed as previously detailed[12,13] by quantitation of the transfer frequency of Lucifer yellow CH dye to directly adjacent neighboring cells following the single cell microinjection.

Results and Discussions. Analogous to the prior observations with oleyl amides, a wide range of arachidonyl secondary and tertiary amides were found to be potent inhibitors of rat glial gap junction-mediated dye transfer (Table 1). Because the assay essentially provides results of dye transfer or no dye transfer, IC_{50} determinations are not possible and the agents often exhibit sharp activity changes over a small concentration range. In the examination of the oleic acid derivatives, most were ineffective at 50 μM and only a select set of past potent inhibitors exhibited activity at this concentration under our assay conditions. In addition, only the arachidonyl amides corresponding to most potent oleyl amide derivatives were examined in the present study. Although this was not established as carefully in the arachidonyl amide series as the oleyl series[13] by examining the effects at lower concentrations, the loss of activity with the larger secondary or tertiary arachidonyl amides indicates that the analogous smaller secondary or tertiary substituents are favored. Thus, like the observations made with oleyl amide derivatives, a wide range of arachidonyl amides bearing sterically undemanding amide substituents provide effective gap junction inhibitors.

The mechanism and role of gap junction inhibition in the expression of the biological properties of oleamide and anandamide is not yet clear.[3,12,13,15,21] However, it is interesting that the less sterically demanding arachidonamides, like oleamide and its derivatives, are less active or inactive at the CB receptors while others

including the *N*-propyl and *N*-butyl amides have greater affinity than anandamide.[3] These distinctions between the trends observed in the CB receptor binding and the gap-junction inhibition may prove useful in distinguishing the origin of the biological effects of both oleamide and anandamide.

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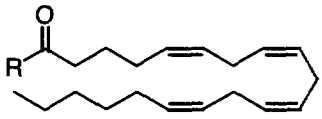
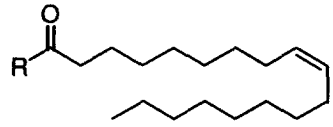
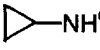
Table 1. Gap junction inhibition





agent (R)	% inhibition		% inhibition[a]	
	50 μM	20 μM	50 μM	20 μM
NH_2	100%	90% (arachidonamide)	100%	100% (oleamide)
OH	0%	0% (arachidonic acid)	0%	0% (oleic acid)
$MeNH$[b]	100%		100%	70-80%
Me_2N[b]	100%		100%	70-85%
$EtNH$[b]	100%		100%	50-60%
Et_2N[b]	100%		100%	15-25%
 N[d]	100%		100%	0%
$CH_2{=}CHCH_2NH$[c]	90%		100%	
$PrNH$[b]	100%		90-100%	50-70%
Pr_2N[e]	10%		nd	
i-$PrNH$[b]	100%		100%	
 NH[e]	100%		100%	
i-$PrNMe$[e]	100%		100%	
$BuNH$[b]	10%		100%	
PhNH	nd		0%	
$Ph(CH_2)_3NH$	nd		0%	
HONH	nd		100%	
$MeONMe$[e]	100%		100%	100%
$HOCH_2CH_2NH$[a]	100%	35% (anandamide)	100%	0%
$(HOCH_2CH_2)_2N$[a]	0%	0%	8%	0%
$HOCH_2CH_2CH_2NH$[a]	nd		100%	

[a]Taken from ref 13. All compounds exhibited the reported or expected characterization properties (NMR, IR, HRMS). Characterization may be found in [b]ref 18, [c]ref 19, and [d]ref 20 or [e]provided upon request.

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