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MINIREVIEW

N-Acyl Amino Acids (Elmiric Acids): Endogenous Signaling Molecules with Therapeutic Potential

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ABSTRACT

The subject of *N*-acyl amino acid conjugates has been rapidly growing in recent years, especially with regard to their analgesic and anti-inflammatory actions. The field comprises a large family of lipid signaling molecules whose importance is only now being fully realized. The most widely studied member is *N*-arachidonoyl glycine (NAGly), which differs structurally from the endocannabinoid anandamide (*N*-arachidonoyl ethanolamide) by a single oxygen atom even as the two are metabolically related. Topics that are covered in this minireview are: biosynthetic pathways for *N*-acyl amino acids, receptors for *N*-acyl amino acids, physiologic actions of *N*-acyl amino acids, pharmacological effects of *N*-acyl amino acids, and molecular mechanisms believed to be responsible for their effects. On the subject of mechanisms, we propose several possibilities whose basis is the currently available information. Four putative pathways can be suggested: 1) inhibition of fatty acid amide hydrolase–induced increases in anandamide or 2-arachidonoyl glycerol (2-AG) levels, resulting in analgesic activity; 2) binding to GPR18, initiating the production of anti-inflammatory eicosanoids (specifically, the data suggest roles for 15-deoxy-$\Delta^{12,14}$-prostaglandin-J_2 and lipoxin A_4, both of which are potent inflammation-resolving molecules); 3) inactivation of T-type Cav3 channels; and 4) inhibition of the GLYT2 glycine transporter. Each pathway would produce analgesic effects. Also, the *N*-acyl amino acids do not bind to either cannabinoid or opioid receptors, thus reducing adverse actions and making them good templates for novel drug candidate molecules.

Introduction

A class of *N*-acyl amino acids in which long-chain fatty acids covalently coupled to amino acids by an amide bond are sometimes called elmiric acids and have emerged as an important family of endogenous signaling molecules (Bradshaw and Walker, 2005) that regulate pain and inflammation (Huang et al., 2001; Burstein et al., 2007; Zurier and Burstein, 2016). The existence of these endogenous substances was first suggested in 1997, (Burstein et al., 1997) and later, synthetic examples were shown to exhibit anti-inflammatory and analgesic activity in mice (Burstein et al., 2000). Subsequent studies identified several naturally occurring *N*-acyl amino acids in rat brain extracts (Huang et al., 2001). Their actions include analgesia (Huang et al., 2001; Vuong et al., 2008; Jeong et al., 2010), anti-inflammatory effects (Burstein et al., 2007), selective inhibition of cancer cell proliferation (Burstein and Salmonsen, 2008), vasodilation (Parmar and Ho, 2010), cell migration (McHugh et al., 2010, 2012a), and calcium ion mobilization (Kohno et al., 2006; Ross et al., 2009). To date, about 70 naturally occurring members as well as several synthetic analogs of the *N*-acyl amino acid family have been identified (Burstein et al., 2007). These comprise various combinations of amino acids linked to long-chain acids by an amide bond (Table 1). For most of these molecules little is known about their biologic activity. An earlier review covered some of these topics and included the related acyl neurotransmitters (Connor et al., 2010); these were briefly updated in a later review (Burstein, 2014).

Burstein SMA, Pearson W, Rooney T, Yagen B, Zipkin R and Zurier A. (1997) Abstract. Studies with analogs of anandamide and indomethacin., in *Symposium on Cannabinoids,* p 31, International Cannabinoid Research Society, Burlington, VT.

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ABBREVIATIONS: 2-AG, 2-arachidonoyl glycerol; AM-251, 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-(1-piperidyl)pyrazole-3-carboxamide; BK, big potassium; CB1, cannabinoid receptor 1; CBD, cannabidiol; CNS, central nervous system; CoA, coenzyme A; COX, cyclooxygenase; ERK, extracellular signal-regulated kinase; FAAH, fatty-acid amide hydrolase; HEK, human embryonic kidney; GABA, γ-aminobutyric acid; GPCR, G protein-coupled receptor; HU-210, 1,1-dimethylheptyl-11-hydroxy-tetrahydrocannabinol; IOP, intraocular pressure; LOX, lipoxygenases; NA, *N*-arachidonyl; NAGly, *N*-arachidonyl glycine; NASer, *N*-arachidonoyl-L-serine; NCXpm, plasma membrane Na^+-Ca^{++} exchanger; OLGly, oleoyl glycine; PCR, polymerase chain reaction; PGJ, 15-deoxy-$\Delta^{12,14}$-prostaglandin-J_2; siRNA, small-interfering RNA; SR144,528, 5-(4-chloro-3-methylphenyl)-1-[(4-methylphenyl)methyl]-*N*-[(1S,2S,4R)-1,3,3-trimethylbicyclo[2.2.1]heptan-2-yl]-1H-pyrazole-3-carboxamide; THC, tetrahydrocannabinol; TRPV1, transient receptor potential cation channel subfamily V member 1.

TABLE 1
Examples of endogenous *N*-acyl amino acid conjugates
Seventy conjugates have been identified in a variety of species (Leishman et al., 2016). General structure: R_1CONHR_2.

Chemical Name	Acyl Group (R_1)	Amino Acid (R_2)
Palmitoyl glycine	(16:0)	gly
Oleoyl glycine	(18:1)	gly
Arachidonoyl glycine	(20:4)	gly
Palmitoyl L-alanine	(16:0)	L-ala
Oleoyl L-alanine	(18:1)	L-ala
Arachidonoyl L-alanine	(20:4)	L-ala
Arachidonoyl GABA	(20:4)	GABA
Stearoyl phenylalanine	(18:0)	phe
Oleoyl phenylalanine	(18:1)	phe
Arachidonoyl phenylalanine	(20:4)	phe
Docosahexaenoyl phenylalanine	(22:6)	phe
Palmitoyl proline	(16:0)	pro
Palmitoyl serine	(16:0)	ser
Stearoyl serine	(18:0)	ser
Oleoyl serine	(18:1)	ser
Linoleoyl serine	(18:2)	ser
Arachidonoyl serine	(20:4)	ser
Palmitoyl tryptophan	(16:0)	trp
Palmitoyl tryptophan	(16:0)	trp
Stearoyl tyrosine	(18:0)	tyr
Arachidonoyl tyrosine	(20:4)	tyr
Docosahexaenoyl tyrosine	(22:6)	tyr
Palmitoyl valine	(16:0)	val

Even though the *N*-acyl amino acids have been known for some time, their roles in cells have only recently become appreciated. They bear many similarities to anandamide, one of the important endocannabinoids, both in chemical structure and in biologic activity. Moreover, they may be metabolically interrelated either directly or indirectly. It is of interest that the levels of *N*-acyl amino acids in rat brain are severalfold higher than that of anandamide (Burstein, 2008). The first to be discovered (Burstein et al., 1997) and the most widely studied acyl amino acid conjugate, *N*-arachidonyl glycine (NAGly) is shown in Fig. 1 and is found in rat brain, spinal cord, and other tissues, where it occurs in amounts greater than the closely related endocannabinoid anandamide (arachidonoyl ethanolamide) (Huang et al., 2001). A preliminary report suggested that NAGly possesses analgesic properties but lacks the psychotropic activity of the cannabinoid receptor 1 (CB1)–active cannabinoids (Burstein et al., 1997). It has been shown that NAGly does not bind to the cannabinoid receptors CB1 or CB2 (Sheskin et al., 1997); however, it appears to activate the orphan G protein-coupled receptor (GPCR) GPR18 (Kohno et al., 2006). Table 2 shows a list of some of the actions of NAGly, which along with other actions will be described in more detail in subsequent sections of this review.

Biosynthetic Pathways for *N*-Acyl Amino Acids

The biologic origin of NAGly is not completely understood; however, two possible biosynthetic pathways have been proposed and data supporting the existence of each have been reported (Burstein et al., 2000; Huang et al., 2001). Using anandamide radiolabeled in the ethanolamine moiety, Burstein et al. (2000) showed that Chang hepatocytes incubated with this precursor produced a radiolabeled peak that chromatographically comigrated with NAGly in several thin-layer chromatography systems. This suggested that NAGly may, under some conditions, be generated by oxidative metabolism of anandamide (Fig. 2). A second pathway, shown in Fig. 3, that involves condensation of arachidonyl coenzyme A (CoA) with glycine, was proposed by Huang et al. (2001), in whose work the process is mediated by a subcellular rat brain preparation. To support this suggestion, they used deuterium-labeled precursors and demonstrated the synthesis of deuterated NAGly by mass spectrometric analysis. Both pathways may operate depending on the specific set of circumstances. Also, Bradshaw et al. (2009) reported evidence that fatty-acid amide hydrolase (FAAH) plays a role in NAGly metabolism; however, such a role appears to be one of anabolism. Additionally, their data suggest that anandamide may serve as a precursor for NAGly, which differs from anandamide only by the oxidation state of the carbon beta to the amido nitrogen.

Other studies found that cytochrome c catalyzes the synthesis of NAGly from arachidonoyl CoA and glycine in the presence of hydrogen peroxide. Hemoglobin and myoglobin were much less effective in mediating the synthesis of NAGly compared with cytochrome c (McCue et al., 2008). It was subsequently reported that cytochrome c also promotes the formation of *N*-arachidonoyl serine, *N*-arachidonoyl alanine, and *N*-arachidonoyl γ-aminobutyric acid from arachidonoyl CoA and the respective amino acids (McCue et al., 2009). Interestingly, it was discovered that arachidonoyl CoA and ethanolamine react spontaneously to form anandamide, independent of cytochrome c and hydrogen peroxide.

Further evidence for the existence of the two pathways was recently reported (McHugh et al., 2012a). When d_4 anandamide was incubated with HEC-18 endometrial cells, two isotopic forms of NAGly were isolated. The d_4 anandamide product was labeled on the ethanolamine part of the structure. The incubation products were d_0-NAGly and d_2-NAGly. The first has to result from the general pathway shown in Fig. 3, and the second has to be generated by the oxidation pathway in Fig. 2.

In addition, the characterization of human glycine *N*-acyltransferase-like 2 (hGLYATL2) has been published (Waluk et al., 2010). This is a member of a family of four putative glycine-conjugating enzymes that synthesizes various *N*-acyl amino acids promoted the conjugation of oleoyl-CoA, arachidonoyl-CoA, and other medium- and long-chain acyl-CoAs specifically to glycine. Its expression pattern, with high levels of *N*-acyl glycines in skin and lung, suggested to the authors a role for these molecules in barrier function/immune response.

In prokaryotic cells, *N*-acyl amide synthase genes are enriched in gastrointestinal bacteria, and it was shown that

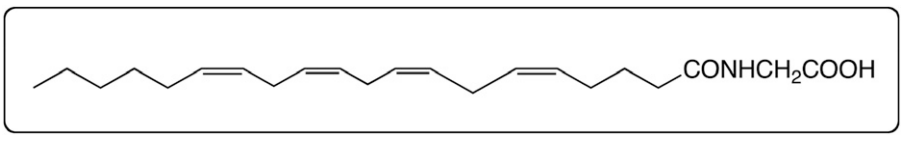

Fig. 1. The structure of *N*-arachidonoyl glycine (NAgly; *N*-[1-oxo-5Z,8Z,11Z,14Z-eicosatetraenyl]-glycine).

TABLE 2
Some of the actions of NAGly

Response	Potency	Reference	System
PTX inhibition of cAMP levels	10 nM	Kohno et al. (2006)	GPR18-transfected CHO cells
Resolution of inflammation	3 μM	Burstein et al. (2011)	RAW264.7 cells
Cell migration	100 nM	McHugh et al. (2010)	BV-2 microglia
Leukocyte migration	0.3 mg/kg	Burstein et al. (2011)	Peritonitis in mice
Inhibition of glycine transport	3.4 μM	Edington et al. (2009)	Frog oocyte GLYT2
Induction of cell migration	44 nM	McHugh et al. (2012a)	Human endometrial HEC-1B cells
Macrophage apoptosis	10 μM	Takenouchi et al. (2012)	RAW264.7 cells
Modulates synaptic transmission	30 μM	Jeong et al. (2010)	Rat spinal cord slices
Vasorelaxant	30 μM	Parmar and Ho (2010)	Rat mesenteric artery
Inhibition of T-type Ca channels	1 μM	Barbara et al. (2009)	tsA-201 cells
Neuropathic pain model	700 nM	Vuong et al. (2008)	Rat paw withdrawal threshold
Elevation of anandamide levels	10 mg/kg	Burstein et al. (2002)	Rat blood
Elevation of anandamide levels	10 μM	Burstein et al. (2002)	RAW264.7 cells

their products interact with GPCRs that regulate gastrointestinal physiology (Cohen et al., 2017). Analysis of one effector gene family (Cbeg12), recovered from the libraries of three patients, found that it encodes for the production of *N*-acyl-3-hydroxypalmitoyl-glycine (commendamide) (Cohen et al., 2015). Commendamide activates the GPCR G2A/GPR132, which has been implicated in disease models of autoimmunity and atherosclerosis. The authors suggested that their findings indicate "a possible small-molecule therapeutic modality (microbiome-biosynthetic gene therapy)."

Receptors for *N*-Acyl Amino Acids

NAGly and the GLYT2 Receptor. Glycinergic neurotransmission is impaired in subjects suffering from chronic pain (Vandenberg et al., 2014). Extracellular glycine levels in the central nervous system are regulated by the glycine transporters GLYT1 and GLYT2. It has been reported that NAGly is an endogenous inhibitor of GLYT2 with little or no effect on GLYT1 and is analgesic in rats with inflammatory pain (Edington et al., 2009). They also reported that, in addition to NAGly, NA-D-Ala and *N*-arachidonyl (NA)–γ-aminobutyric acid (GABA) interact with the EL2 (extracellular loop) of GLYT2. Interestingly, NA-L-Ala showed little activity. It is of further interest to note that in an anti-inflammatory model, a D-Ala acyl amino acid was more active than its L-Ala stereoisomer (Burstein et al., 2012). This suggests that there may be some similarity in the underlying molecular mechanisms responsible for these two actions because of their similar stereo selectivities for GLYT2 inhibition and resolution of inflammation.

A later report suggested a molecular mechanism for the spinal analgesic actions of NAGly (Jeong et al., 2010). They found that NAGly enhances glycinergic synaptic transmission within the lumbar superficial dorsal horn, which is an important center for the integration of ascending pain information in the central nervous system (CNS). Nociceptive transmission within the superficial dorsal horn may involve multiple mechanisms, including glycine transporters and possibly *N*-methyl-D-aspartate receptors, in addition to T-type Ca^{++}-channels that were reported to mediate NAGly's peripheral analgesic actions (Barbara et al., 2009; Ross et al., 2009).

GPR18: A Putative Receptor for NAGly. There are no reports on the binding of any of the *N*-acyl amino acids to receptor molecules, since, apparently, binding assays have not yet been developed. Thus, no receptor-binding antagonists have been validated. However, there is substantial functional evidence to suggest a role for GPR18 in mediating some of the actions of NAGly. Stimulation of GPR18 with NAGly has

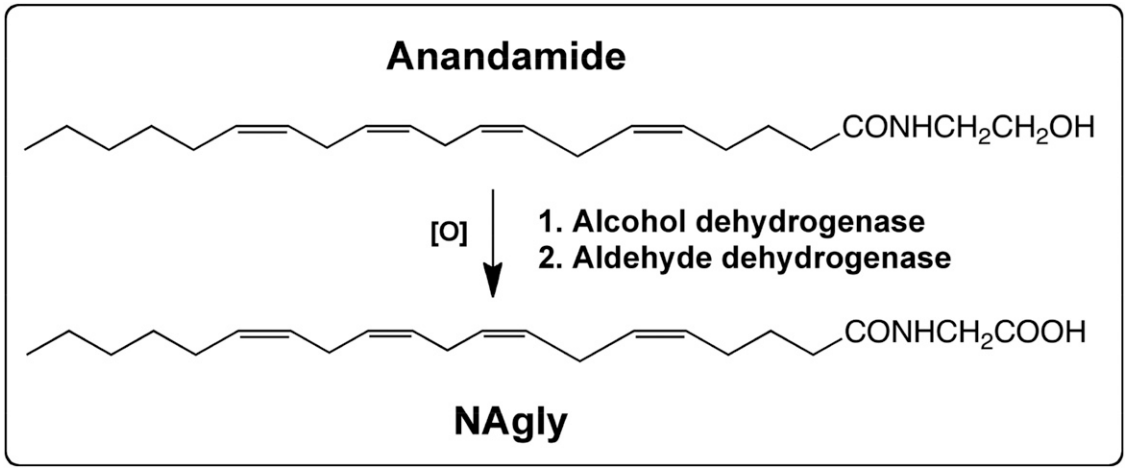

Fig. 2. NAGly from anandamide. This particular *N*-acyl amino acid can be generated by a two-step oxidative metabolism of the endocannabinoid anandamide. The transient aldehyde intermediate has not been isolated.

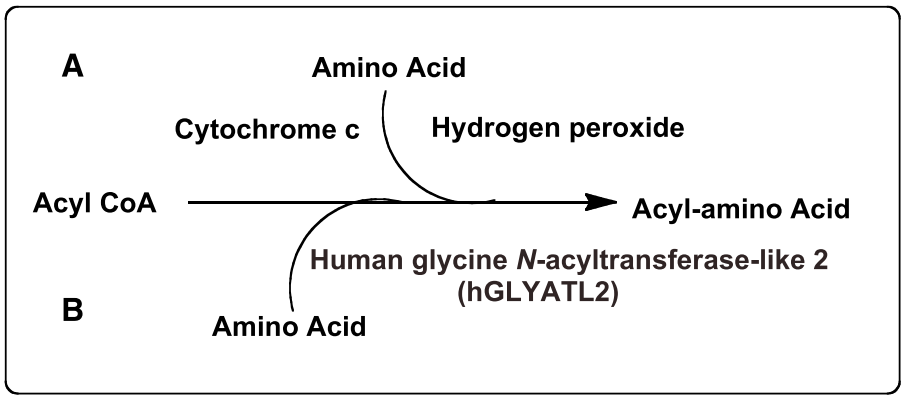

Fig. 3. Two general pathways for a variety of acyl amino acids. (A) Cytochrome c catalyzes the synthesis of an *N*-acyl amino acid from a fatty acid coenzyme A and glycine in the presence of hydrogen peroxide. (B) The same transformation is mediated by an *N*-acyl transferase called human glycine *N*-acyltransferase-like 2 or hGLYATL2.

functional consequences (Kohno et al., 2006; McHugh et al., 2010, 2012a,b). On the other hand, reports by Yin et al. (2009) and Lu et al. (2013) claim anomalous findings as to the responsiveness of NAGly and GPR18. Thus, the role of GPR18 following ligand exposure remains somewhat unclear. Since anandamide and tetrahydrocannabinol (THC) are full agonists of GPR18, it has been suggested that it may be considered a novel cannabinoid receptor (Alexander, 2012).

GPR18, a member of the GPCR superfamily, was discovered and its sequence reported in 1997 (Gantz et al., 1997). In a subsequent report, Kohno et al. (2006) de-orphanized GPR18 with the identification of NAGly as a putative ligand on the basis of functional responses. This was the only "hit" out of a group of 200 bioactive lipids screened by them, suggesting a high degree of structural selectivity. As described by the authors, "GPR18 was cloned on the basis of degenerate-oligonucleotide polymerase chain reaction (PCR) analysis of HUT 102 cells using primers designed from the conserved regions of the human chemokine receptor. GPR18 was expressed significantly in lymphoid cell lines, but not in nonlymphoid hematopoietic cell lines. The expression of the GPR18 gene was higher in peripheral lymphocyte subsets [CD4(+), CD4(+)CD45RA(+), CD4(+)CD45RO(+), CD8(+), and CD19(+)] than in monocytes and lymphoid cell lines, and was increased after stimulation with phytohemagglutinin. By screening using a lipid library, NAGly induced increased Ca^{++} concentration in GPR18-transfected cells, which was significantly greater than that in mock-transfected cells. NAGly also inhibited forskolin-induced cAMP production in a pertussis toxin-sensitive manner in GPR18-transfected CHO cells." This was the first study to demonstrate that NAGly may be a natural ligand for GPR18.

In a concentration-dependent manner, NAGly increased intracellular calcium and extracellular signal-regulated kinases (ERKs) 1/2 phosphorylation for human embryonic kidney (HEK)-293/GPR18 cells (Console-Bram et al., 2014). In the presence of NAGly, the initial rise in intracellular calcium was blocked by either $G\alpha_q$ or $G\alpha_{i/o}$ inhibition. Pertussis toxin and *N*-arachidonoyl-L-serine inhibited the ERK1/2 phosphorylation and inhibited the NAGly-induced increases. The authors concluded that GPR18 activation involves several signal transduction pathways, suggesting the possibility of biased agonism.

McHugh et al. (2010) found that NAGly is an extremely active recruiter of BV-2 microglia and its effects can result in anti-inflammatory actions in the brain. They examined the relationships between NAGly, Abn-CBD, the unidentified "Abn-CBD" receptor, GPR18, and BV-2 microglial migration. They reported that NAGly at low nanomolar concentrations promotes proliferation and activation of mitogen-activate protein kinases in BV-2 microglia and HEK-293/GPR18 cells; cellular responses correlated with microglial migration. These authors suggested that GPR18-NAGly may be a lipid-based signal employed by the CNS to actively recruit microglia to sites of injury. Their hypothesis was that NAGly initiates directed microglial migration in the CNS through activation of GPR18. Small-interfering (si)RNA knockdown data supporting this hypothesis was reported (McHugh et al., 2012b). BV-2 microglia transfected with GFP+ GPR18 siRNA had reduced GPR18 mRNA levels and immunocytochemical staining. Cell migration induced by 1 μM concentrations of NAGly was significantly decreased in GFP+ cells.

NAGly stimulates apoptosis in mouse RAW cells; a more robust increase was seen in cells with higher expression levels of GPR18 (Takenouchi et al., 2012). This response could be reduced by pretreatment of these cells with either pertussis toxin or GPR18-specific siRNA. Data were also reported on the signaling pathway involved in the observed NAGly-induced apoptotic effect, providing additional support for GPR18's role in an action of NAGly.

Another example of GPR18's mediation was described in a study on the migration of human endometrial HEC-1B cells (McHugh et al., 2012a). Migration stimulated by NAGly showed a "bell-shaped" dose-response effect with a maximum at 10 nM. Estradiol and THC were used as comparison compounds and, interestingly, all three had the same type of response curve, albeit maxima differed. NAGly gave the most robust effect, being 300% of the estradiol response. The presence of GPR18 was shown by PCR measurement of GPR18 mRNA.

Evidence against GPR18 Involvement. A library of lipid molecules not containing NAGly was screened against a series of orphan receptors using the PathHunter β-arrestin assay system (Yin et al., 2009). It was found that GPR18 did not mediate a response with any of the lipids in this library. Subsequently, NAGly, which was not in the library, was tested and found to be inactive in the β-arrestin model.

A more recent study concluded that either NAGly is not an agonist for GPR18 or that GPR18 signaling involves noncanonical pathways (Lu et al., 2013). This study was done in a native neuronal system, namely, GPR18 heterologously expressed in rat sympathetic neurons, and researchers measured opening of

N-type (Cav2.2) calcium channels. They reported no evidence for the activation of $Ga_{i/o}$-protein signaling after NAGly treatment of neurons with heterologously expressed GPR18. Other downstream effectors of $G\alpha_{i/o}$-coupled receptors, G protein-coupled inwardly rectifying potassium channels and adenylate cyclase, were also not affected. Lu et al. (2013) concluded that "the disconnect between localization of GPR18 and endogenous NAGly supports the presence of another receptor for NAGly, which is responsible for signaling in neurons."

Using a previously described assay (Grimsey et al., 2008), it was found that GPR18 undergoes rapid constitutive receptor membrane trafficking (Finlay et al., 2016). Moreover, in several functional responses, under a variety of conditions, a lack of ligand-mediated responses was observed in cells treated with NAGly. One explanation that was offered was that GPR18 might only be functional when coexpressed with another receptor. Interestingly, there has been some discussion in the literature about the possible occurrence of cannabinoid receptor homo or hetero dimerization (Mackie, 2005). If such hetero dimers involving GPR18 exist, they may provide an explanation for the divergent reports from several laboratories on GPR18 functional activities.

GPR92 as a Receptor for NAGly. NAGly is an endogenous ligand for the G-protein coupled receptor GPR92, along with farnesyl pyrophosphate. Dorsal root ganglia express GPR92, and NAGly increased intracellular calcium levels in these neurons, indicating a role for NAGly in the sensory nervous system through the activation of GPR92 (Oh et al., 2008). NAGly activated the $G_{q/11}$-mediated signaling pathway; however, other ligands, namely, farnesyl pyrophosphate and lysophosphatidic acid, were able to activate both $G_{q/11}$- and G_s-mediated signaling pathways. GPR92 mRNA is also expressed in peripheral tissues such as spleen, stomach, small intestine, and kidney. It was proposed that NAGly may have a role in the sensory nervous system resulting from activation of GPR92.

Physiologic Actions of *N*-Acyl Amino Acids

A selected group of *N*-acyl amino acids and some of their biologic actions is shown in Table 3. Although not complete, it illustrates the diversity of structures and activities characteristic of this family of molecules.

FAAH Inhibition. NAGly is a potent inhibitor of FAAH, the enzyme primarily responsible for the degradation of the endocannabinoid anandamide (Grazia Cascio et al., 2004). Several *N*-acyl amino acids were synthesized and their FAAH inhibitory activity measured. The potencies were found to depend on both species and chemical structure. Thus, in human-derived FAAH, the following rank order was observed: NAIle > NAGly = NA-L-Ala > NA-D-Ala. In the rat, the order was: NAGly > NA-L-Ala > NA-D-Ala = NAIle, and in the mouse it was: NAGly > NA-D-Ala > NA-L-Ala = NAIle. The effects of the FAAH inhibitor URB 597 on the endogenous levels of *N*-acyl amino acids in mouse brain were reported (Han et al., 2013). Anandamide and *N*-arachidonoyl serine levels showed a dose-dependent increase after systemic administration of URB 597, whereas NAGly and NAGABA were significantly decreased after treatment. NAAla and 2-AG were not altered after URB 597 treatment.

Control of Tissue Anandamide Concentrations. Data have been reported suggesting that NAGly may serve as an endogenous regulator of tissue anandamide concentrations (Burstein et al., 2002). In rats, oral administration of NAGly at a dose of 10 mg/kg increased blood concentrations of anandamide 9-fold, as shown by mass spectrometry. This response may result from inhibition of FAAH, causing a reduction in the hydrolytic cleavage of anandamide. A similar effect was seen in vitro when RAW 264.7 mouse macrophage (RAW) cells were exposed to d_8-NAGly. The anandamide measured had no deuterium, indicating that it did not come from the administered d_8-NAGly. Thus, it is reasonable that NAGly, and possibly other *N*-acyl amino acid conjugates, may influence physiologic levels of anandamide. Although speculative, these conjugates may provide scaffolds for improved pharmacological agents able to raise anandamide levels.

FAAH-Dependent NAGly Action Mediated by GPR18. There is evidence for a functional GPR18-based signaling system in the murine anterior eye (Caldwell et al., 2013). The regulation of lipids in the eye by FAAH and *N*-arachidonoyl phosphatidyl ethanolamine phospholipase, mRNA expression of these enzymes, and their role in diurnal regulation of intraocular pressure (IOP) in mice were measured (Miller

TABLE 3

Examples of some of the actions of *N*-acyl amino acids

Some of these actions are discussed in detail in sections *Physiologic Actions of N-Acyl Amino Acids* and *Mechanisms Responsible for the Effects of N-Acyl Amino Acids*.

Chemical name	Selected Signaling Actions
N-Palmitoyl glycine	Production of NO (Rimmerman et al., 2008)
N-Oleoyl glycine	Increased adipogenesis (Wang et al., 2015)
N-Arachidonoyl glycine	Signaling at GPR18 (McHugh et al., 2014)
N-Palmitoyl L-alanine	Inhibits cancer cell proliferation (Burstein and Salmonsen, 2008)
N-Arachidonoyl L-alanine	Allosteric modulation of GlyR (Yévenes and Zeilhofer, 2011)
N-Arachidonoyl GABA	Inhibits pain (Huang et al., 2001)
N-Oleoyl phenylalanine	Improves glucose homeostasis (Long et al., 2016)
N-Arachidonoyl-L-serine	Regulate homeostasis of the vascular system (Milman et al., 2006)
N-Arachidonoyl-L-serine	Allosteric modulation of GlyR (Yévenes and Zeilhofer, 2011)
N-linoleoyl-D-alanine	Increased production of PGJ (Burstein et al., 2012)
N-Arachidonoyl-isoleucine	Inhibition of human FAAH (Grazia Cascio et al., 2004)
N-Palmitoyl tyrosine	Antiproliferative action in cancer cells (Burstein and Salmonsen, 2008)
Arachidonoyl GABA	Modulates vascular tone (Parmar and Ho, 2010)
N-Stearoyl-L-threonine	Active against brain ischemia (Yao et al., 2009)
N-Palmitoyl tryptophan	Inhibits Sendai virus fusion to liposomes (Epand et al., 1998)

et al., 2016). The results support FAAH-dependent NAGly action at GPR18 as a physiologic basis for the diurnal variation of IOP in mice. It was suggested that GPR18 might serve as a target for the development of novel ocular hypotensive medications.

Effects Involving the Eicosanoid Superfamily. The first step in the biosynthesis of all of the eicosanoids is the release of free arachidonic acid from phospholipid storage sites. A large number and variety of agents activate specific phospholipases to initiate this process. Among the agonists are both phytocannabinoids such as THC and endocannabinoids such as anandamide. Treatment of RAW cells with NAGly results in a rapid and robust release of free arachidonic acid that can serve as a precursor for eicosanoid synthesis, such as prostaglandins, lipoxins, and endocannabinoids (Burstein, 2008, 2011).

Cyclooxygenases (COX) and lipoxygenases (LOX) oxygenate polyunsaturated fatty acids, such as arachidonic acid, to generate eicosanoid signaling molecules. Similarly, COX-2, but not COX-1, selectively metabolizes NAGly to PGH_2-Gly and hydroxyeicosatetraenoic HETE-Gly (Prusakiewicz et al., 2002). NAGly is naturally present at significant levels in many of the same mammalian tissues that express COX-2, suggesting a possible physiologic role for this action. It may be a strategy for regulating NAGly levels; moreover, little is known about possible activities of these novel eicosanoid-amino acid conjugates. It would be of interest to see whether other *N*-arachidonoyl amino acids are also substrates for COX-2. In a second pathway, the *N*-acyl amino acids were efficiently oxygenated by 12S- and 15S-LOX (Prusakiewicz et al., 2007). It was observed that the 15S-LOX produced positional specificity, whereas 12S-LOX gave products that were oxygenated at both the 12 and 15 positions. Kinetic data were also reported for these transformations.

A group of *N*-acyl amino acids was screened for effects on prostaglandin production in vitro (Burstein et al., 2007). Little or no effect was seen on the levels of PGE_2; however, moderate to robust increases in 15-deoxy-$\Delta^{12,14}$-prostaglandin-J_2 (PGJ) were obtained in RAW cells treated with several of the *N*-acyl amino acids. The PGJ responses of the group were compared with responses from an in vivo anti-inflammatory assay, namely, phorbol ester-induced mouse ear edema. The following rank orders of activity were observed:

PGJ increase: NA-I-Ala = NAGly > LINGly > NA-d-Ala > PALGly,

Ear Edema reduction: NA-I-Ala > NAGly > LINGly > NA-d-Ala > PALGly.

The most active substance in both assays was NA-L-Ala and the least active was *N*-palmitoyl glycine (PALGly), suggesting that there is structural specificity in the anti-inflammatory actions of the *N*-acyl amino acids. The difference in activity between the D and L stereoisomers of the alanine conjugates indicates the possibility of a protein receptor.

Inflammation Resolving Action of NAGly. NAGly may act to resolve chronic inflammation, and data has been presented to support this hypothesis (Burstein et al., 2011). In an established assay, the mouse peritonitis model, agents reduce migration of inflammatory leukocytes following injection of proinflammatory agents into the peritoneal cavity. A single NAGly treatment (1.2 mg/kg by mouth) resulted in a significant 70% reduction of peritoneal cells. This demonstrated both the efficacy and bioavailability of *N*-acyl amino acids in this model. Experiments with GPR18-transfected HEK-293 cells support a role for this receptor. Levels of two inflammation-resolving eicosanoids, PGJ and lipoxin A_4, were increased in a dose-related manner. Also, PCR analysis for GPR18 mRNA in four cell types showed a near perfect correlation ($r^2 = 0.98$) with PGJ levels after NAGly treatment.

***N*-Linked Amino Acid–Linoleic Acid Conjugates.** The linoleic acid analog of NAGly, linoleoyl glycine (LINGly), was examined for its inflammation-resolving actions (Burstein et al., 2012). In the mouse peritonitis assay, at a dose of 0.3 mg/kg given orally, it reduced leukocyte migration by 75%. Harvested peritoneal cells showed increased PGJ production in LINGly-treated mice versus vehicle-treated mice when incubated ex vivo. A small group of conjugates was tested in RAW cells at 10 μM for PGJ-stimulating activity. The following rank order of activity was observed.

N-LIN-d-Ala > N-LINGly > NAGly > N-LIN-d-Tyr > N-LIN-I-Ala > N-LIN-d-Phe.

The high degree of stereospecificity between the D and L isomers of alanine strongly suggested receptor mediation in this action. Also of interest, and somewhat unexpected, was the finding that *N*-linoleoyl glycine was more active than *N*-arachidonoyl glycine. This may have important implications for future studies in drug discovery.

Inactivation of T-Type Cav3 Channels. T-type calcium channels have important roles in cell excitability and calcium signaling and are involved in a number of physiologic functions. Moreover, they have been suggested as therapeutic targets in several diseases such as epilepsy, insomnia, neuropathic pain, cancer, and hypertension. Using standard whole-cell voltage clamp electrophysiology techniques, it was shown that NAGly (10 μM/l) inhibited Cav3 channels expressed in HEK-293 cells by approximately 50% (Ross et al., 2009). I(Ca) (voltage-gated calcium channel current) in mouse sensory neurons was also inhibited. It was concluded that NAGly is a member of a new family of endogenous T-type I(Ca) modulators.

In a similar study, it was reported that *N*-acyl amino acids, including NAGly, reversibly inhibited Cav3.1, Cav3.2, and Cav3.3 currents in T-channels expressed in tsA-201 cells (Barbara et al., 2009). In vivo this was expressed as strong thermal analgesia that was abolished in Cav3.2 knockout mice. Thermal nociception was determined by measuring paw withdrawal latency following immersion of the right hind paw into a 46°C water bath. As a result of these findings, efforts have been made to discover synthetic T-channel inhibitors (Cazade et al., 2014). At the molecular level, there appears to be a common mechanism for the actions of synthetic T-channel inhibitors such as TTA-A2 and several endogenous lipids. These include NAGly, NASer (*N*-arachidonoyl-L-serine), anandamide, NADA (*N*-arachidonoyl dopamine), NATau (*N*-arachidonoyl taurine), and NA-5HT (*N*-arachidonoyl serotonin). Binding of [3H]TTA-A1 (a radiolabeled derivative of TTA-A2) to membranes prepared from HEK-293 cells stably expressing Cav3.3 was reported; membranes from wild-type cells were inactive (Cazade et al., 2014). Thus, TTA-A2 may be a useful agent in future studies on the effects of lipids on T-channels.

The above topics are the subject of a recent review (Chemin et al., 2014).

Activation of N-Type Ca^{++} Channels. N-type Ca^{++} channels (Cav2.2) in rat sympathetic neurons were reported to be activated by *N*-arachidonoyl L-serine (Guo et al., 2008). Several other *N*-acyl amino acids were less active than NA-L-serine; these included NAGly and NA-L-Ala. The canonical G protein-coupled receptors did not appear to be involved in this action; however, no other mechanism was proposed. A comparison with free arachidonic acid action on Ca^{++} is discussed in view of the possible metabolism of NA-L-Ser to the free acid and serine.

NAGly as a Novel Insulin Secretagogue. A number of molecules can influence the level of glucose-stimulated insulin secretion by ultimately triggering an intracellular Ca^{++} flux. A primary β-cell-based functional assay was used to measure this response to NAGly in pancreatic islets from male ICR mice (Ikeda et al., 2005). On the basis of their findings, they suggested that NAGly increases $[Ca^{++}]i$ in β-cells through voltage-dependent Ca^{++} channels in a manner independent of the vanilloid receptor VR1.

***N*-Oleoyl Phenylalanine Improves Glucose Homeostasis.** Beige fat cells contain an enzyme, PM20D1, that can conjugate fatty acids to amino acids (*N*-acyl amino acids); it is bidirectional and can also promote hydrolysis in vitro. It was reported that *N*-acyl amino acids bind mitochondria and are able to act as endogenous activators of uncoupling protein 1-independent respiration (Long et al., 2016). The administration of *N*-acyl amino acids to mice improved glucose homeostasis and increased energy expenditure and represents a family of molecules that can regulate energy homeostasis. Among the group that was studied, *N*-oleoyl phenylalanine was the most active. The authors suggested that such compounds may be effective in "the treatment of human obesity and diabetes, and to modulate thermogenesis more generally."

Effects of NAGly on Store-Operated Ca^{++}. NAGly is highly effective in specifically inhibiting store-operated Ca^{++} (SOCE) in a variety of cell types by reducing the interaction of stromal interacting molecule 1 with Orai1, the pore-forming subunit of SOCE channels (Deak et al., 2013). This inhibition occurs in a time- and concentration-dependent manner and is reversible. Of interest is the comment that the IC_{50} of NAGly on SOCE is the same as the effective concentration of NAGly needed to initiate the resolution of chronic inflammation (Burstein et al., 2011).

Effects on Microglial-Neuronal Communication. NAGly-GPR18 signaling may be a target for microglial-neuronal communication (McHugh et al., 2012a, 2014). NAGly at 10–100 nM affected the production of five cytokines, namely, Axl, CD40, IGF-I, OPN, and Pro-MMP-9 in BV-2 microglia in a concentration-dependent manner by treatment with NAGly at these concentrations. Interestingly, prior exposure of the cells to cannabidiol (CBD) antagonized these effects. These studies concluded that NAGly "tightly integrates microglial proliferation, recruitment, and adhesion with neuron-glia interactivity and tissue remodeling." And further, that "a greater understanding of this system is essential to improving our ability to therapeutically monitor and manage dysregulated microglial activity."

Effect of NAGly on Endothelial Electrical Signaling. NAGly inhibits plasma membrane Na^{+}-Ca^{++} exchanger (NCXpm)-mediated ion current in a concentration-dependent and G protein-independent fashion (Bondarenko et al., 2013). NAGly inhibited membrane hyperpolarization in histamine- and ACh-stimulated cells by reduction of NCXpm-mediated Ca^{++} inflow. However, in unstimulated cells, NAGly produced hyperpolarization by direct stimulation of big potassium (BK) Ca channels. NAGly can exert an inhibitory effect on both the forward and reversed NCXpm. In de-endothelialized NA-precontracted rat aortic segments, NAGly blocks the contraction that is, in part, controlled by NCXpm-mediated Ca^{++} entry. The authors suggest that these data show that the effect of NAGly on NCXpm is either a result of its interaction with the NCX protein itself or modifications of the lipid bilayer. These findings may have significance for "the development of novel effective approaches for the management of cardiovascular disorders."

Increased Lipid Accumulation and Adipogenic Genes in 3T3-L1 Cells. *N*-Oleoyl glycine (OLGly) increased adipogenic genes (PPARγ and aP2) in a concentration- (20–50 μM) and time-dependent manner in 3T3-L1 cells (Wang et al., 2015). Lipid accumulation and triglyceride levels were also elevated. Interestingly, OLGly increased the mRNA expression of the CB1 receptor. Moreover, pretreatment with SR141716, a CB1 antagonist, reduced these effects of the *N*-acyl amino acid, suggesting a role for CB1. The stimulation of adipogenesis, activation of insulin-mediated Akt signaling pathway, and inactivation of FoxO1 were blocked by wortmannin, a specific PI3K/Akt inhibitor, suggesting a role for an Akt signaling pathway. It was concluded that OLGly could enhance insulin sensitivity, resulting in suppression of obesity and diabetes. This possibility is somewhat dampened by the relatively high concentrations needed to produce the reported data.

OLGly has a somewhat related action on energy homeostasis (Wu et al., 2017). The effects of NAGly, NASer, and OLGly on food intake (6 mg/kg intraperitoneally) and $[Ca^{++}]i$ of agouti-related protein (AgRP) neurons (20 μM) were studied to identify the role of these *N*-acyl amino acids in energy homeostasis. AgRP increases food intake and decreases metabolism and energy expenditure and is a potent and long-lasting stimulator of appetite. OLGly-induced activation of neuronal AgRP was completely eliminated by the CB1-specific antagonist AM251. Also, it was reported that NAGly and NASer were inactive, demonstrating structural specificity in this model.

Regulation of Endocannabinoid Production and Pain. Bradshaw et al. (2006) measured the levels of the pain-modulatory lipids anandamide, 2-AG, NAGly, NAGABA, and *N*-arachidonoyl dopamine in seven different brain areas. They reported that the levels are regulated differently between genders and that most of these differences vary with changes during the estrous cycle. The levels of NAGly in males were significantly lower in striatum compared with females in diestrus and night proestrus. Likewise, NAGABA levels in males were significantly lower compared with females in metestrus, diestrus, night proestrus, and estrus. No cycle-dependent gender differences in 2-AG, anandamide, or *N*-arachidonoyl dopamine were observed. The data obtained from this study will be helpful in future studies on the relationships between endocannabinoids, hormonal milieu, and pain.

Pharmacological Effects of *N*-Acyl Amino Acids

NAGly in a Rat Model of Neuropathic Pain. The effect of NAGly in a rat model of neuropathic pain was compared with the CB1/CB2 agonist HU-210 (Vuong et al., 2008). Mechanical

allodynia was induced by partial ligation of the sciatic nerve and NAGly (700 nmol) was administered by intrathecal injection. The resulting anti-allodynia response was dose-dependent and was not reduced by pretreatment with either the CB1 or the CB2 receptor antagonists AM251 and SR144,528. Administration of HU-210, but not NAGly, caused a decrease in rotarod latency, indicating the absence of CNS-mediated responses by the latter. These findings suggest the possible use of *N*-acyl amino acids for the treatment of neuropathic pain.

Actions of NAGly in Ocular Functions. The NAGly receptor GPR18 is expressed in several areas in the eye: namely, the ciliary epithelium, the corneal epithelium, and in the trabecular meshwork (Caldwell et al., 2013). NAGly was found in the mouse eye at a level comparable to that seen in the brain, suggesting a role in ocular function. NAGly reduced IOP in mice, and the GPR18 antagonist O-1918 blocked this effect. NAGly reduced IOP independently of CB1, CB2, or GPR55. These findings indicate that there may be a functional GPR18-based signaling system in the murine anterior eye. Thus, *N*-acyl amino acids might be developed as novel ocular hypotensive medications.

Evidence has been reported that suggests a role for non-CB1/CB2 receptors in mediating the relaxant actions of NAGly on retinal endothelin-1 (ET-1)-induced vasoconstriction (MacIntyre et al., 2014). GPR18 mRNA and protein were seen in the retina; in addition, immunohistochemistry showed that GPR18 was localized to the endothelium of retinal vessels. NAGly inhibited ET-1-induced vasoconstriction in retinal arterioles by an endothelium-dependent mechanism that involves small-conductance calcium-activated potassium channels. Vasorelaxation was completely blocked by apamin, an agent that inhibits small-conductance calcium-activated potassium channels. Also, it was shown that neither CB1 nor CB2 plays a role in the regulation of retinal microvasculature tone.

Cancer: Antiproliferative and Cytotoxic Effects of *N*-Acyl Amino Acids. *N*-acyl amino acids affect the proliferation of RAW cells (Burstein et al., 2007). A library of 20 *N*-acyl amino acids was screened for antiproliferative activity in an in vitro assay using the mouse leukemic monocyte-macrophage cell line RAW264.7. This line was established from an ascites induced in a male mouse by intraperitoneal injection of Abelson leukemia virus. The library was composed of a series of conjugates of long-chain fatty acids with either glycine or L-alanine that were administered at 0.1, 1.0, and 10 μM concentrations. Many of the conjugates produced around 90% reduction in cell numbers in a dose-related manner. Oleoyl glycine was the most active with oleoyl L-alanine being slightly less active. Although inconclusive for cancer therapy, these findings suggested that further study was warranted. Such a follow up study was, in fact, published shortly thereafter (Burstein and Salmonsen, 2008). Several cell lines were studied, including HTB-125 (normal human breast cells), HTB-126 (human breast cancer cells), HeLa (cervical cancer cells), WI-38 (human embryonic lung cells), RAW264.7 (mouse macrophage tumor cells), and RBL-2H3 (rat basophilic leukemia cells). Most informative were the HTB lines that were obtained from the same donor, which could be considered a matched pair. *N*-palmitoyl tyrosine demonstrated complete specificity of antiproliferative action at a concentration of 10 μM and was also highly efficacious. These data suggest the possibility that Paul Ehrlich's concept of a "silver bullet" may some day be realized in cancer chemotherapy. Curiously, NAGly was reported to have only a weak effect on the viability of human colorectal carcinoma Caco-2 cells (Gustafsson et al., 2009). It was less potent than anandamide in inhibiting [^{3}H]-thymidine incorporation. At 30 μM, NAGly produced 21% and 40% decreases in [^{3}H]-thymidine incorporation and nucleic acid content, respectively.

NAGly Modulates Vascular Tone. NAGly produces a vasorelaxant action by the activation of BK Ca in rat small mesenteric arteries. (Parmar and Ho, 2010). It may activate an unknown $G_{i/o}$-coupled receptor, stimulating endothelial release of NO that in turn activates BK Ca in smooth muscle. Moreover, NAGly might also activate BK Ca through $G_{i/o}$- and NO-independent mechanisms. Pretreatment with antagonists for CB1, CB2, and TRPV1 receptors, or inhibitors of FAAH and COX had no effect. NASer and NAGABA were also examined and compared with NAGly. They induced iberiotoxin- and L-NAME-sensitive relaxations in a descending order of potency: NAGABA > NAGly > NASer.

The action of NAGly on blood pressure, regional blood flow, and vascular tone in the rat superior mesenteric artery was studied in rats (Al Suleimani and Al Mahruqi, 2017). More specifically, the roles of the endothelium, NO, Na^+/Ca^{++} exchanger, and Ca^{++}-sensitive K^+ channels, as well as of the transient receptor potential cation channel subfamily V member 1 (TRPV1) receptors, CB1 and CB2, GPR55, and PPARγ were also studied. In addition, the effect of NAGly on carbachol- and phenylephrine-induced tone change was measured. Vasorelaxation was largely, if not solely, endothelium-dependent, and mediated by multiple mechanisms involving activation of an endothelial site distinct from the cannabinoid receptors. Most of the effects were produced by NAGly concentrations of 30 μM, suggesting pharmacological rather than physiologic actions.

A different *N*-acyl amino acid, *N*-arachidonoyl-L-serine (NASer) causes endothelium-dependent vasodilation of rat mesenteric arteries and abdominal aorta and stimulates phosphorylation of p44/42 mitogen-activated protein kinase and protein kinase B/Akt in cultured endothelial cells. It also inhibits lipopolysaccharide-induced formation of tumor necrosis factor-α in a murine macrophage cell line and in wild-type mice, as well as in CB1 or CB2 knockout mice (Milman et al., 2006). NASer was isolated from bovine brain; however, there was no mention of other *N*-acyl amino acids such as NAGly, which is probably present in greater abundance. The rationale for focusing on NASer was stated to be its structural similarity to anandamide, but NAGly differs from anandamide by only one oxygen atom whereas NASer has major structural differences compared with anandamide. Thus, in real life, NASer may not be a major factor in the physiologic regulation of vascular tone.

Subsequent studies of NASer on human brain endothelial cells described effects of this *N*-acyl amino acid on the cytoskeleton (actins) and on the signal transduction pathways that are involved (Kino et al., 2016). Interestingly, data were reported indicating the involvement of three receptors, CB1, CB2, and TRPV1. Evidence was also described that NASer is a modulator of Rho kinase in this model. These actions could result in functional responses such as vasodilation, proliferation, and movement.

Neuroprotective Effect Against Brain Ischemia. The neuroprotective effects of a group of 17 *N*-acyl amino acids against brain ischemia on rat cerebral slices was evaluated (Yao et al., 2009). Ischemia was induced by oxygen-glucose deprivation (OGD). The most active molecules were *N*-stearoyl-L-tyrosine,

N-stearoyl-L-serine, and *N*-stearoyl-L-threonine, which exhibited good activity protecting rat brain slices against OGD as well as hydrogen peroxide insult at the range of 1–10 μM.

The neuroprotective effects of *N*-arachidonoyl-L-serine in vivo in a closed head injury model of traumatic brain injury were later published (Cohen-Yeshurun et al., 2011, 2013). The authors suggest that this response involves "ERK and Akt phosphorylation and induction of their downstream anti apoptotic pathways." They also believe that these "protective effects are related mostly to indirect signaling via the CB2R and TRPV1 channels but not through CB1 or GPR55 receptors."

In a follow up study, the proneurogenic properties of *N*-arachidonoyl-L-serine in vitro and in vivo after traumatic brain injury were examined. The treatment outcomes reported were a reduction in lesion volume and an improvement in neurobehavioral function.

Antiviral Activity. A group of *N*-palmitoyl amino acids were tested for their inhibitory action against Sendai virus fusion to liposomes composed of egg phosphatidylethanolamine and 5 mol % of the ganglioside GD1a (Epand et al., 1998). It was reported that several of the *N*-palmitoyl amino acids were effective inhibitors of the fusion of Sendai virus to liposomes as measured by the R18 assay. The most active molecule was *N*-palmitoyl tryptophan; its inhibitory action against Sendai virus was confirmed by showing inhibition of Sendai-mediated cytopathic effects studied in tissue culture. The *N*-palmitoylated amino acids of Ala, Val, Ile, Leu, and Gly had no significant action on viral fusion up to mole fractions as high as 0.2. The *N*-acyl amino acids PALTyr and PALTrp displayed marked inhibition of fusion at mole fractions as low as 0.005.

Mechanisms Responsible for the Effects of *N*-Acyl Amino Acids

Membrane Effects. Studies were reported that may provide a thermodynamic and structural basis for defining the functional roles of *N*-acyl glycines in the tissues where they occur. For example, these findings could be important for understanding their interactions with constituents of biomembranes, such as integral membrane proteins, and with membrane lipids, such as phospholipids and cholesterol (Reddy et al., 2014). Experiments were done to characterize a series of *N*-acyl glycines with regard to their thermotropic phase behavior and structure by differential scanning calorimetry (DSC) and X-ray diffraction. The DSC results indicated that the thermodynamic parameters ΔH_t and ΔS_t showed a linear dependence on the chain length both in the dry state and upon hydration.

Molecular Events. Figure 4 shows a scheme for several putative mechanisms that may mediate the anti-inflammatory and analgesic actions of NAGly. One pathway involves the inhibition of FAAH resulting in increased anandamide and 2-AG levels followed by activation of CB1, and as a result analgesia is produced (Huang et al., 2001; Grazia Cascio

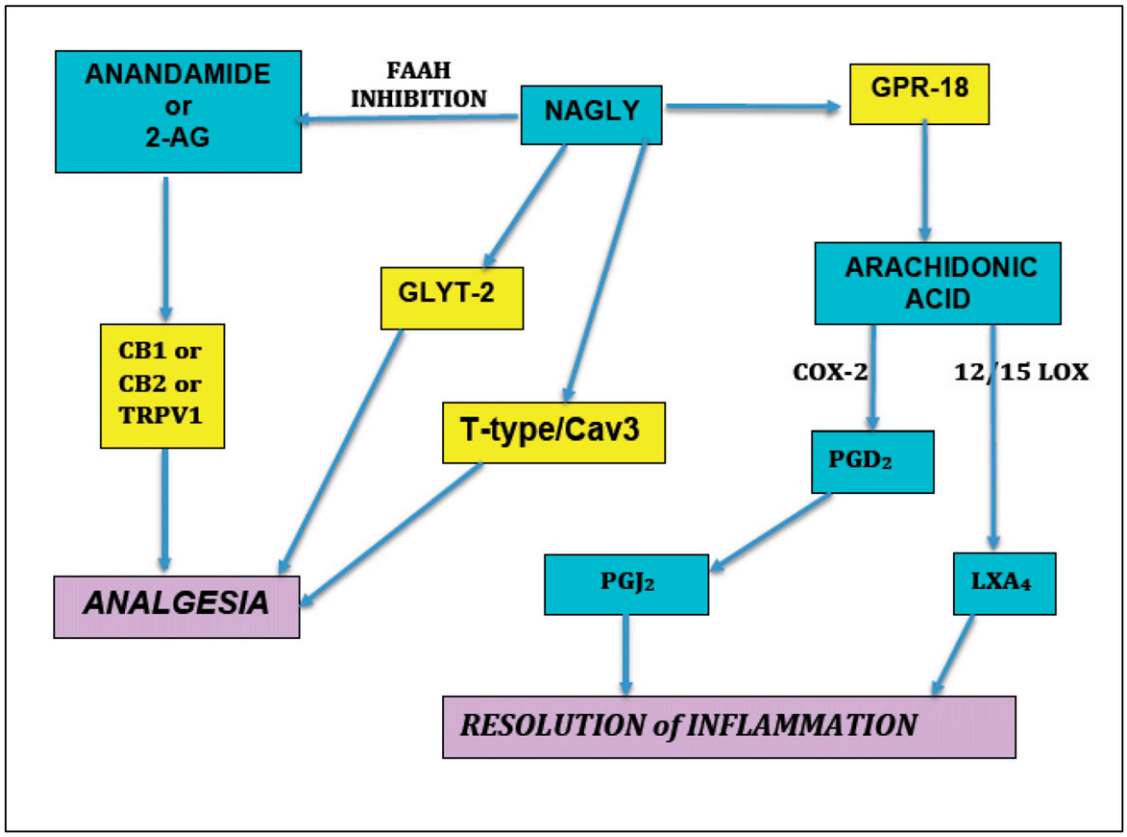

Fig. 4. Putative mechanisms that have been suggested to mediate the anti-inflammatory and analgesic actions of NAgly. Inflammation-resolving action is initiated by the activation of the GPCR GPR18 followed by the release of free arachidonic acid. Subsequent action by either COX-2 or 12, 15 lipoxygenases results in the synthesis of PGJ_2 and/or lipoxin A_4, both of which are potent inflammation-resolving agents. Analgesic action of NAGly may be a result of its anandamide-elevating effect caused by the inhibition of FAAH. In addition, NAGly is an endogenous inhibitor of the glycine transporter GLYT2. NAGly-induced thermal analgesia is abolished in Cav3.2 knockout mice, illustrating yet another mechanism.

et al., 2004). Activation of CB2 and TRPV1 may also occur in some circumstances. Other pathways leading to analgesic activity involve mediation by GLYT-2 (Edington et al., 2009) or T-type/CAV2 (Barbara et al., 2009).

Yet another pathway is initiated by the activation of the orphan receptor GPR18 when NAGly is an endogenous ligand (Kohno et al., 2006; McHugh et al., 2012a). The initial response is the release of free arachidonic acid from pools of the esterified acid (Burstein, 2008; Burstein et al., 2011). The free acid is the precursor for most of the eicosanoid family of signaling molecules. At this point, the mechanism may follow one or both of two highly regulated directions, one controlled by COX-2 (Burstein et al., 2007) and the other by 12/15 lipoxygenase (Burstein et al., 2011). Either of these two pathways stimulate the synthesis of inflammation-resolving signaling molecules (Fullerton and Gilroy, 2016).

Conclusions and Future Directions

The discovery of the analgesic effects of the *N*-acyl amino acids has opened an entirely new approach toward the treatment of pain. Because of their serious side effects, the existing analgesics have limited utility in pain therapy, particularly in chronic pain. NAGly has shown efficacy in several preclinical pain models. For example, in the rat sciatic nerve ligation model, the authors concluded that "it was unlikely that the observed effects of NAGly were due to direct, or even indirect activation of cannabinoid receptors" (Vuong et al., 2008). So, despite its structural similarity to the endocannabinoid anandamide, NAGly appears to act through a novel receptor, possibly GPR18. The pursuit of this hypothesis is hampered by the lack of a sensitive binding assay. Preliminary data supporting the feasibility of a GPR18 binding assay using high-specific-activity $^{3}H_{8}$-NAGly as the ligand has been reported (Burstein, 2014). A future goal should be to develop a sensitive and specific radioligand binding assay for GPR18. These considerations would also apply to the treatment of chronic inflammation with such molecules.

It is unlikely that NAGly itself will ever become a drug candidate; however, a more potent and stable synthetic analog could become a safe and efficacious agent for dealing with the above mentioned major unmet medical needs. Some of the data reported for other members of the *N*-acyl amino acid family suggest that analogs of NAGly could become potential drug candidates. Thus, a serious effort should be made to search for novel GPR18 agonists with greater potency and metabolic stability. Libraries of such analogs could be easily generated by conjugating a series of synthetic amino acids with various long-chain fatty acids. An added outcome from such research could be the discovery of a specific antagonist for the GPR18 receptor. Such drug candidates would have a high safety level, since NAGly does not bind to either of the cannabinoid or any of the opioid receptors, thereby reducing the possibility of adverse actions.

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