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PERSPECTIVE

Selective Ligands for Lysophosphatidic Acid Receptor Subtypes: Gaining Control over the Endothelial Differentiation Gene Family

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In this issue of *Molecular Pharmacology*, Heise et al. (2001) report their results with derivatives of *N*-acyl ethanolamide phosphate that display subtype-selective agonist and antagonist properties for the endothelial differentiation gene (EDG) family of lysophosphatidic acid (LPA) receptors (Heise et al., 2001). This report follows a publication by Fischer et al. (2001) in the October issue of *Molecular Pharmacology* reporting on the LPA receptor subtype-selective antagonist properties of short-chain phosphatidic acids. These compounds might be the prototypes of tools long awaited by researchers attempting to unravel the physiological and pathophysiological roles of growth factor-like phospholipids.

Lysophosphatidic acid (1-acyl-2-*sn*-glycero-3-phosphate) and sphingosine-1-phosphate (S1P) have generated considerable interest among cell biologists and pharmacologists since the early 1990s because of their ability to evoke robust Ca^{2+} responses and changes in cell shape at nanomolar concentrations (for review, see Moolenaar, 1999; Pyne and Pyne, 2000; Tigyi, 2001). This interest has been augmented by the ability of LPA and S1P to elicit mitogenic responses and to sustain the survival of cultured cells at micromolar concentrations. The biological effects of these lipids are utilized—unwittingly by most—when serum, which contains LPA in the 10 μM range (Baker et al., 2001) and S1P in the 100 nM range (Yatomi et al., 1995), is added to culture media. The biological responses to LPA and S1P are consistent with those of ligands acting through specific G protein-coupled receptors. However, because of the hydrophobic nature of these ligands, the application of radioligand binding assays for the detection of specific receptors has been challenging. This is one of the factors that have made the molecular cloning of the phospholipid growth factor receptors difficult. However, Hecht et al. (1996) identified LPA as a ligand for a G-protein-coupled receptor they isolated from the ventricular zone of the developing mouse brain. The cloned and overexpressed *vzg-1* gene mediated serum-induced retraction of neurites in cortical neurons, a characteristic response elicited by LPA application in PC12 (Tigyi and Miledi 1992) and N1E115 (Jalink et al., 1993) neuroblastoma cells noted earlier. *Vzg-1* was later named EDG2 (Contos et al., 2000) because it was shown to be highly homologous to a family of G-protein-coupled orphan receptors. The first member of this family was cloned by Hla and Maciag as a phorbol ester-induced early response gene in vascular endothelial cells (Hla and Maciag 1990); hence, it was named EDG1 for endothelial differentiation gene-1. In 1998, Lee et al. reported that S1P was an endogenous ligand for EDG-1. After this report, several groups have identified other members of this family, including the genes for the LPA-specific EDG4 (An et al., 1998) and EDG7 (Aoki et al., 2000) receptors and the S1P-specific EDG3/5 (An et al., 1997), EDG-6 (Gräler et al., 1998), and EDG8 receptors (Im et al., 2000). This year, the IUPHAR Nomenclature Committee proposed a new nomenclature, based on the receptor's natural ligand and the chronological order of its discovery, to replace the colloquial EDG terminology (Table 1).

Fischer et al. (1998) have shown that most cell types coexpress multiple subtypes of the EDG family receptors. Moreover, there are hints that the EDG family might not represent the only receptors for LPA and S1P (Guo et al., 1996; Hooks et al., 2001). The complexity of the pharmacology of these receptors has delayed the identification of ligands with receptor-selective antagonist and agonist properties. Such compounds are essential for the continued advancement of the field. Because the coexpression of multiple receptor subtypes occurs in most cell types, knock-out mouse models have shown minimal phenotypic alterations (Contos et al., 2000;

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ABBREVIATIONS: EDG, endothelial differentiation gene; LPA, lysophosphatidic acid; S1P, sphingosine-1-phosphate; DGPP, dioctylglycerol pyrophosphate; NAEPA, *N*-acyl ethanolamide phosphate.

TABLE 1
Nomenclature of phospholipid growth factor receptors

Old Name	IUPHAR Name	Natural Ligand	Agonist Preference	Antagonist Preference
EDG2	LPA_1	LPA	VPC31143 > *sn*1LPA > *sn*2LPA	VPC12249 ≫ DGPP(8:0)~PA(8:0)
EDG4	LPA_2	LPA	*sn*1LPA > *sn*2LPA	N.A.
EDG7	LPA_3	LPA	*sn*2LPA > *sn*1LPA, unsaturated	DGPP > PA(8:0)~VPC12449
EDG1	$S1P_1$	S1P	S1P > S1P-phosphonate	N.A.
EDG5	$S1P_2$	S1P	S1P > SPC	N.A.
EDG3	$S1P_3$	S1P	S1P > SPC	N.A.
EDG6	$S1P_4$	S1P	S1P > SPC	N.A.
EDG8	$S1P_5$	S1P	S1P > SPC	N.A.

N.A., not yet available; SPC, sphingosylphosphorylcholine.

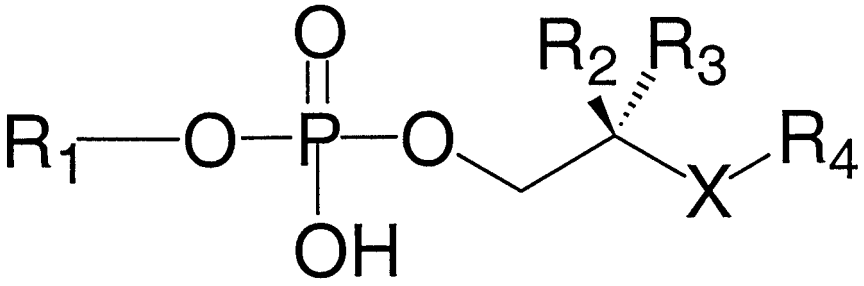
Ishii et al., 2001), although one of them resulted in embryonic lethality (Liu et al., 2000). Thus, the two recent *Molecular Pharmacology* publications examining subtype-specific ligands are likely to generate additional studies that explore the application of receptor subtype-selective agonists and antagonists to different cell systems and animal models.

The two articles describe two different sets of compounds with differing receptor subtype-selectivity (Table 2). The work of Fischer et al. (2001), based on earlier structure-activity studies (Jalink et al., 1995; Bandoh et al., 2000), has shown that truncating the length of the *sn*-1 hydrocarbon chain results in a differential loss of agonist properties toward the three LPA receptors, and that adding an *N*-octyl chain to the *sn*-2 position of the glycerol backbone converts the resulting dioctyl phosphatidic acid into an antagonist. Based on computational studies and experimental validation of $S1P_1$ and LPA_1 receptor models, our group, in collaboration with Dr. Parrill's group (Parrill et al., 2000a,b; Sardar et al., 2001; Wang et al., 2001) has characterized the essential requirement for ionic interactions between LPA and two conserved positively charged residues, R3.28 (Ballesteros and Weinstein, 1995) in the third, and K(R)7.36 in the seventh transmembrane domains of the LPA receptors. To explore the effects of extra charges in the polar headgroup, we have tested dioctylglycerol pyrophosphate (DGPP), which was found to have slightly better antagonistic properties over that of dioctyl phosphatidic acid. These short chain phosphatidates showed preferential competitive inhibition of the LPA_3 receptor subtype (K_i ~100 nM) over LPA_1 (K_i ~6.6 μM) and LPA_2, which is neither inhibited nor activated by these two compounds up to 10 μM concentration. Fischer et al. (2001) also examined a variety of LPA- and S1P-responsive cell types that express different combinations of LPA and S1P receptors to support a selective inhibitory action by these two compounds on the LPA_3 receptor subtype. DGPP potently inhibited LPA-induced shape changes in human platelets and was without effect on PAF- and thromboxane-induced response (Rother et al., 2001). One concern with the use of DGPP is that an earlier report found that it activated cPLA2 and ERK1/2 in P388D1, a macrophage cell line (Balboa et al., 1999).

The article by Heise et al. (2001) in this issue of *Molecular Pharmacology* has explored a different lead compound, *N*-acyl ethanolamide phosphate (NAEPA), which was originally synthesized by Sugiura et al. (1994) in Dr. Hanahan's group. In retrospect, these seminal observations represented a breakthrough that was not fully appreciated by researchers in the field despite work by our laboratory with that of Dr. Bittman's group (Bittman et al., 1996; Liliom et al., 1996) and that of the Lynch laboratory supported by Dr. McDonald's groups (Hooks et al., 1998, 2001; Im et al., 2000). Based on the pioneering work of Dr. Hanahan's group (Sugiura et al., 1994), we synthesized the enantiomers of *N*-palmitoyl-L(D)-serine-phosphoric acid and *N*-palmitoyl-L(D)-tyrosine-phosphoric acid, which inhibit LPA responses in *Xenopus laevis* oocytes (Liliom et al., 1996; Hooks et al., 1998), platelets (Sugiura et al., 1994; Siess et al., 1999), and endothelial cells (Rizza et al., 1999; Siess et al., 1999). However, *N*-palmitoyl-L(D)-serine-phosphoric acid at micromolar concentrations was found to be a weak LPA mimetic on LPA_1 and LPA_2 receptors heterologously overexpressed in HTC4 cells, tempering further testing of the compound (An et al., 1998). In this issue, Dr. Lynch's team reports on the characterization of eight enantiomeric pairs of novel NAEPA derivatives. Heise et al. (2001) identified three important trends in the receptor selectivity of NAEPA derivatives: 1) the receptors show a stereoselectivity, although not an absolute one, for one enantiomer over the other; 2) compounds with short-chain substituents on R3 (Table 2), specifically methyl (VPC12086), methylene-amino (VPC12178), and methylene-hydroxy (VPC31143) analogs, are agonists with a rank-order receptor preference LPA_1 LPA_2 LPA_3; and 3) the enantiomeric pair of benzyl-4-oxybenzyl analogs have different properties. VPC12204 is a weak agonist, whereas VPC12249 was devoid of agonist action and showed a selective competitive inhibitory effect with K_i values of ~130 nM on LPA_1 and ~430 nM on the LPA_3 receptor, with no effect on LPA_2. The latter discovery of receptor subtype selectivity has far-reaching implications for the design of antagonists. It had been well established that LPA-elicited effects lack stereo-specific recognition (Simon et al., 1982; Jalink et al., 1995), because the natural (*R*)-LPA and unnatural (*S*)-LPA are both biologically active. Heise et al. (2001), however, have shown that NAEPA analogs, unlike LPA, are recognized in a stereoselective manner and that this additional interaction unique to NAEPA derivatives can provide the foundation for the design of selective antagonists. These authors also provide data from endogenously LPA-responsive human embryonic kidney 293T cells and from rat arterial blood pressure measurements in support of the pharmacological properties of their analogs.

Why should one care about novel ligands for phospholipid growth factors? LPA receptor agonists are expected to possess antiapoptotic effects (Goetzl et al., 1999; Weiner and Chun 1999) and promote wound healing (Balazs et al., 2001). On the other hand, LPA antagonists have potential applications as inhibitors of inflammation (Rizza et al., 1999), cancer invasiveness (Imamura et al., 1993), and atherogenesis

TABLE 2
Structure-activity relationship of LPA receptor ligands



Compound	R_1	R_2	R_3	X	R_4
LPA 18:1	H	H	OH	CH_2	$OCO(C_{17}H_{33})$
PA (8:0)	H	H	$OCO(C_7H_{15})$	CH_2	$OCO(C_7H_{15})$
DGPP (8:0)	OPO_3	H	$OCO(C_7H_{15})$	CH_2	$OCO(C_7H_{15})$
NAEPA	H	H	H	NH	$CO(C_{17}H_{33})$
NP-ser-PA	H	COOH	H	NH	$CO(C_{15}H_{31})$
VPC12204	H	Benzyl-4-oxybenzyl	H	NH	$CO(C_{17}H_{33})$
VPC12249	H	H	Benzyl-4-oxybenzyl	NH	$CO(C_{17}H_{33})$

(Siess et al., 1999; Hayashi et al., 2001). Ovarian cancer cells produce LPA-like substances that seem to act as paracrine factors to enhance the invasiveness and chemotherapeutic resistance of this cancer (Xu et al., 1995; Frankel and Mills 1996; Pustilnik et al., 1999; Eder et al., 2000; Fang et al., 2000). LPA seems to cause dedifferentiation of vascular smooth muscle cells in vitro only if it contains unsaturated fatty acids, presumably through the LPA_3 receptor subtype; this mimics many of the events that occur during the early stages of atheromatous plaque formation (Hayashi et al., 2001). The inhibitors described by these two groups, some of which are now commercially available, can be tested further in a variety of in vivo model systems. The results of these two studies should therefore provide further insights into the biology and therapeutic applicability of phospholipid growth factor ligands.

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