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Molecular pharmacology of adipocyte-secreted autotaxin

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Abstract

Autotaxin is a type II ecto-nucleotide pyrophosphate phosphodiesterase enzyme. It has been recently discovered that autotaxin also catalyses a lyso-phospholipase D activity. This enzyme probably provides most of the extracellular lyso-phosphatidic acid from lyso-phosphatidylcholine. There is almost no pharmacological tools available to study autotaxin. Indeed, all the reported inhibitors, thus far, are uneasy-to-use, lyso-phosphatidic acid derivatives. Initially, autotaxin was recognized as a phosphodiesterase (NPP2) [Bollen et al., Curr. Rev. Biochem. Biol. 35 (2000) 393–432], based on sequence similarity and enzymatic capability of autotaxin to catalyse ecto-nucleotidase activity. Phosphodiesterase forms a large family of enzymes characterized by a large number of chemically diverse inhibitors. None of them have been tested on autotaxin activity. For this reason, we screened those reported inhibitors, as well as a series of compounds, mostly kinase inhibitor-oriented, on autotaxin activity. Only two compounds of the various phosphodiesterase inhibitors (calmidazolium and vinpocetine) were potent enough to inhibit autotaxin catalytic activity. From the kinase inhibitor library, we found damnacanthal and hypericin, inhibiting phosphodiesterase activity in the 100-μM range, comparable to most of other available phospholipid-like inhibitors.

Keywords: Autotaxin; Lyso-phosphatidylcholine; Inhibition; Purification; Phosphodiesterase

Adipocyte lyso-phospholipase D (lyso-PLD) catalyses the transformation of lyso-phosphatidylcholine (LPC) into lyso-phosphatidic acid (LPA) [1]. LPA is a bioactive phospholipid regulating a wide range of cellular responses (proliferation, survival, motility, ion flux, secretion) through the activation of G-protein-coupled receptors: LPA_1, LPA_2, and LPA_3 [2,3] and two more distantly related receptors: LPA_4 and LPA_5 [4]. Autotaxin has been purified from various sources such as fetal bovine serum [5], human plasma [6] and from adipocytes [7]. ATX catalyses and therefore possesses lyso-phospholipase D activity.

ATX is an extracellular enzyme of 125 kDa. It was first identified as a brain specific membrane glycoprotein belonging to the phosphodiesterase I gene family [8]. There might be several isoforms of this enzyme [9–11]. Recent findings have linked autotaxin and LPA with various physio-pathological processes such as adipocyte function regulation [12,13] and metastasis invasion [14].

Less have been reported so far on the biochemical characteristics and on the pharmacology of this enzyme. LPA and sphingosine 1 phosphate have been reported to have inhibitory activity but no pharmacological applications because the manipulation of these phospholipids is difficult [15]. Indeed, the reported inhibitors of ATX

Abbreviations: LPA, lyso-phosphatidic acid; LPC, lyso-phosphatidylcholine; ATX, autotaxin; Lyso-PLD, lyso-phospholipase D; CM, conditioned medium; CCM, concentrated conditioned media; tlc, thin layer chromatography.

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Table 1
Pharmacology of autotaxin

Inhibitors or substrates	Reference IC_{50}	PDE (IC_{50}, μM)	Lyso-PLD
*p*Nppp (*para*-nitrophenyl phenylphosphonate)		–	3022 ± 150
LPA (oleoyl)	(IC_{50} ~0.1 μM) [15]	0.06 ± 0.01	–
Sphingosine 1-phosphate	(IC_{50} ~0.1 μM) [15]	–	–
8-mBMX (8-methoxymethyl-3-isobutyl-1-methylxanthine)	PDE type I (IC_{50} = 4 μM) [27]	>10000	1200 ± 100
Calmidazolium	PDE I (IC_{50} = 10 nM) [28]	>1000	142 ± 15
Vinpocetine	PDE type I (IC_{50} = 20 μM) [29]	>10000	122 ± 24
EHNA	PDE type II (IC_{50} = 1 μM) [30]	>1000	1050 ± 160
Quizanon	PDE type III (IC_{50} = 600 nM) [31]	4200 ± 250	>10000
Milrinone	PDE III (IC_{50} = 300 nM) [32]	>1000	1290 ± 340
Ro-20-1724	PDE type IV (Ki = 3.1 μM) [33]	>1000	1820 ± 400
Zaprinast	PDE type V (IC_{50} = 450 nM) [34]	1300 ± 100	900 ± 78
4-3′-4′ (Methylenedioxy) benzylamino-6-methoxyquinazoline	PDE V (IC_{50} = 230 nM) [35]	>1000	1480 ± 820
Trequinsin	PDE (substrat: GMPc) (IC_{50} = 300 pM) [36]	>1000	178 ± 79
3-Isobutyl-1,4,3′,4′-(methylenedioxy)benzylamino-6-methoxyquinazoline	cAMP, cGMP phosphodiesterase (IC_{50} = 2–50 μM) [37]	>1000	1390 ± 300
Nitrendipine	No selectif PDE inhibitors [38]	7980 ± 2000	2160 ± 500
Pentoxifylline	No selectif PDE inhibitors [39]	>10000	2340 ± 700
Papaverine	No selectif PDE inhibitors [40]	>1000	337 ± 42
Phenanthroline	Inhibition lyso-PLD (100 μM) [1]	158 ± 46	1090 ± 14
EDTA	Inhibition lyso-PLD (50 μM) [1,41]	117	1000
Ethanol	Activation [1,41]		
ATP		427	3250

All experiments were ran thrice, independently. The values are presented as means ± S.E.M.

are derived from LPA or LPC [16–18]. Furthermore, some of them have been reported as antagonists at the LPA receptors as well [16]. Therefore, our goal in the present paper was to report on our first attempt to characterize autotaxin activity for small molecule inhibitors sensitivity, particularly towards the compounds active on the enzymes of the same family, i.e. phosphodiesterases. Furthermore, we also attempted to find inhibitors distinct from phospholipid derivatives. Since the core activity of phosphodiesterase enzymes is to recognize cAMP and to cleave it, we rationalized to screen compounds known to inhibit enzymes by acting at the level of their nucleotide-binding site, such as compounds inhibiting kinases, most of them being competitive to the ATP-binding site [19]. Furthermore, such libraries of compounds are commercially available. To do so, recombinant human autotaxin was expressed in COS cells, purified and characterized for its lyso-PLD and phosphodiesterase activities. Its characteristics were compared with autotaxin purified from adipocyte-conditioned medium. A couple of new compounds were found to have inhibitory capacities toward autotaxin activity, particularly damnacanthal and hypericin, although these activities remain of poor potency: μM to mM range. The phosphodiesterase inhibitors tested herein were reported with activities toward at least one of the members of this family of enzymes with IC_{50} in the nanomolar range (see Table 1 for details) exemplifying considerable differences in sensitivity toward inhibitors inside the phosphodiesterase family. Nevertheless, they could be used as scaffold to move towards more potent and selective inhibitors.

1. Materials and methods

1.1. Compounds

All chemicals were obtained at the highest purity grade available from Sigma (St Louis, Mo). A library of kinase inhibitors (Biomol Kinase) is provided by Sigma–Aldrich (St. Louis, MO, USA) and contained: PD 98059, RO 31-8220, AG 494, H-7, H-9, AG 370, KN 93, AG 99, AG 18, AG 82, AG 879, AG 183, AG 9, AG 1288, BAY 11-7082, AG 1295, AG 17, RG 14620, PPI, AG 490, AG 1478, AG 825, KN 62, AG