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COMMENTARY

SPHINGOID BASES AS ENDOGENOUS CATIONIC AMPHIPHILIC "DRUGS"

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Many therapeutically effective and clinically useful drugs may be categorized as cationic amphiphilic drugs (CADs†). The classification of CADs is based on their physicochemical properties. As a group, CADs share two distinct domains: a hydrophobic (often aromatic) domain and an ionizable nitrogen atom which can become positively charged (Fig. 1). There has been wide clinical use of CADs in the treatment of mental illness, cardiovascular disease, allergy and inflammation. The CADs include the neuroleptics (phenothiazines, thioxanthenes and butyrophenones), anti-depressants (tricyclic compounds and monoamine oxidase inhibitors), tranquilizers (benzodiazepines), local anesthetics (procaine and its congeners), anti-arrhythmics (e.g. propranolol) and other drugs. The cationic amphiphilic nature of all these drugs was implicated in their propensity in causing lipidosis [1].

Sphingoid bases are natural cationic amphiphilic substances

Sphingoid bases are natural long chain amino bases which constitute the backbone of all sphingolipids (e.g. sphingomyelin, gangliosides) (Fig. 1, top). Sphingoid bases have a typical 2-amino-1,3-diol hydrophilic head structure and a long (usually 15-carbon) hydrophobic alkyl chain (Fig. 1). Naturally occurring sphingoid bases vary in their C1-3 stereoisomeric configuration, presence and configuration of a C4-5 double band, the length of the aliphatic chain and, possibly, the presence of N-methylation. In lyso-sphingolipids, a highly polar headgroup, such as phosphocholine or a glycan, is esterified at the 1-hydroxy position. Hence, by virtue of containing two hydropathically opposed structural domains—a hydrophilic cationic headgroup and a hydrophobic tail—sphingoid bases should be regarded as cationic amphiphilic substances.

Over the past 4 years, sphingoid bases and lyso-sphingolipids have attracted considerable experimental attention. This was primarily because they inhibit the activity of protein kinase C *in vitro* and of cellular events which depend on protein kinase C activation *in vivo* (see Refs. 2 and 3 for review). Hannun *et al.* [4] have proposed that free sphingoid bases, generated through an as yet hypothetical "sphingolipid cycle", may act as intracellular messengers modulating cell function. On the other hand, because they accumulate in affected tissues in the course of sphingolipidoses, lyso-sphingolipids were proposed to play a role in the pathogenesis of these inherited disorders of sphingolipid metabolism [5]. In addition to the inhibitory effect of sphingoid bases and lyso-sphingolipids on protein kinase C, a number of cellular and biochemical effects of sphingoid bases appear not to be mediated by inhibition of this kinase (see Ref. 6 for review). Hence, sphingoid bases seem to have emerged as a new class of putative, lipid-derived bio-regulatory molecules.

Sphingoid bases mimic the biochemical actions of CADs

Recently, in the course of studies on the mechanisms of phospholipase D activation, we discovered that sphingoid bases elicit a rapid and marked increase in the levels of phosphatidic acid [7]. This effect results from both the activation of phospholipase D [7] and the inhibition of phosphatidic acid phosphatase [8]. The high levels of phosphatidic acid thus produced are accompanied by increased levels of the acidic phospholipids for which it is a precursor, i.e. phosphatidylinositol and phosphatidylglycerol [7], and by lower levels of diacylglycerol [7, 8]. The effects of sphingoid bases on phospholipid metabolism are very similar to the effects of CADs, which similarly redirect phospholipid biosynthesis toward the acidic phospholipids [9]. This modulation of phospholipid metabolism, which CADs and sphingoid bases exert in common, probably involves alterations of multiple enzymatic activities. Indeed, compounds from both groups inhibit phosphatidic acid phosphatase activity [8, 10, 11] and inhibit CTP:phosphocholine cytidylyltransferase activity [12, 13]. To further extend the parallelism between CADs and sphingoid bases, we examined the effect of chlorpromazine, a phenothiazine neuroleptic employed in the treatment of schizophrenia, on phospholipase D activity. Indeed, in addition to elevating phosphatidic acid, chlorpromazine stimulated phospholipase D activity as seen by production of its specific product phosphatidylethanol in NG108-15 cells (Lavie Y and Liscovitch M, unpublished results). The sites of action of CADs and sphingoid bases on the enzymatic

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† Abbreviations: CADs, cationic amphiphilic drugs; ECT, electroconvulsive shock treatment; and EGF, epidermal growth factor.

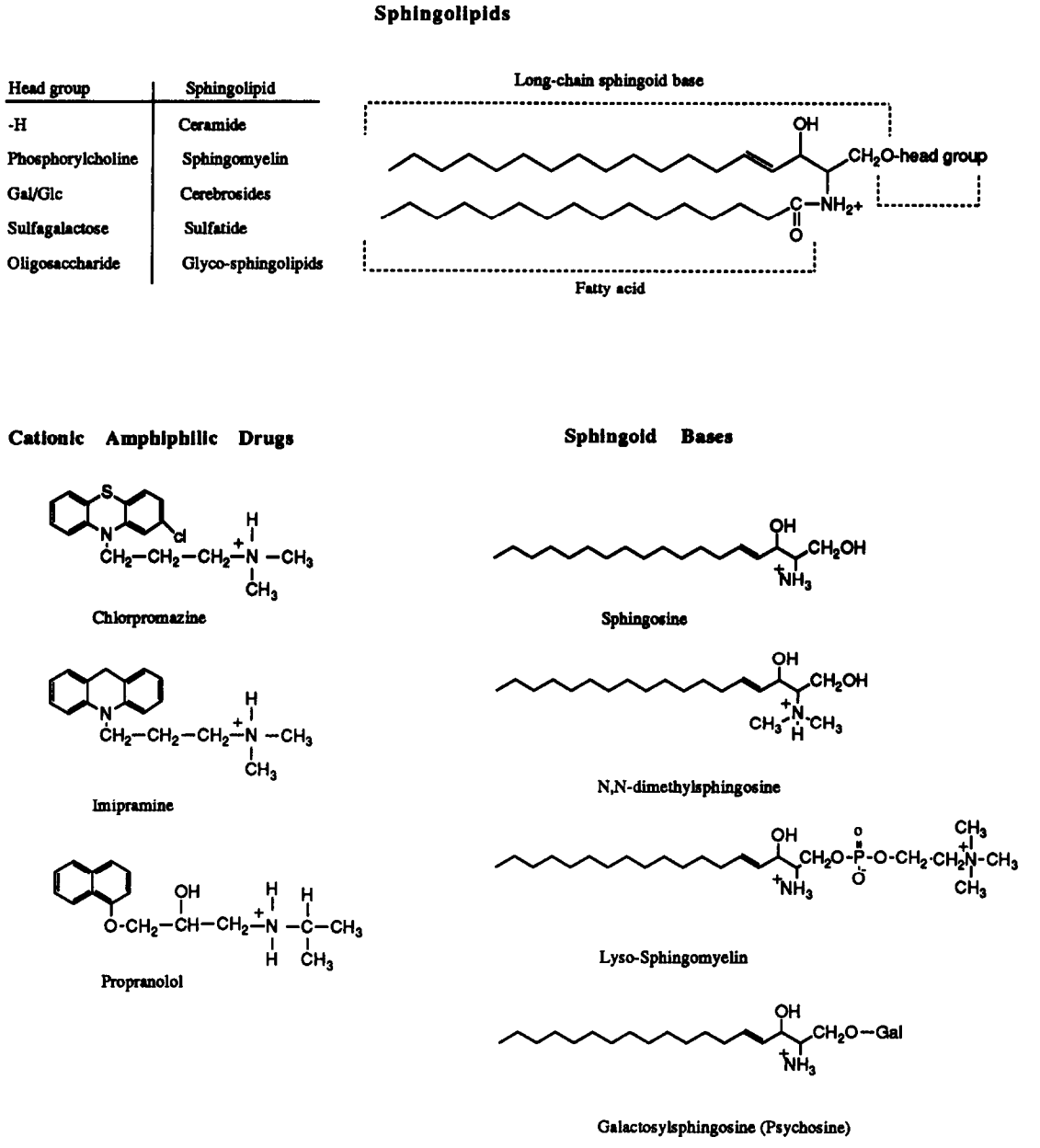

Fig. 1. Structures of sphingolipids, sphingoid bases, lyso-sphingolipids and representative cationic amphiphilic drugs (CADs).

pathways of phospholipid metabolism are shown in Fig. 2. In addition to the parallel effects of sphingoid bases and CADs on phospholipase D, CTP:phosphocholine cytidylyltransferase and phosphatidic acid phosphatase, the sphingoid base sphingosine was also reported to activate diacylglycerol kinase activity [14] and CADs were shown to activate CTP:phosphatidic acid cytidylyltransferase [15]; whether the latter two effects can be mimicked by CADs and sphingoid bases, respectively, remains to be determined.

Prompted by the striking resemblance in the domain structure of sphingoid bases and CADs and their similar effects on phospholipid metabolism, we compared their effects on other biochemical reactions, as reported in the literature (Table 1). Both CADs [16] and sphingoid bases [4, 5] inhibit the activity of protein kinase C. Similarly, compounds from both groups inhibit the activity of Ca^{2+}/calmodulin-dependent enzymes [17, 18]. Tricyclic antidepressants and phenothiazine neuroleptics cause uncoupling of oxidative phosphorylation accompanied by increased oxygen consumption in rat heart mitochondria [19]. By the same token, galactosylsphingosine (psychosine) stimulates mitochondrial respiration in liver and brain while

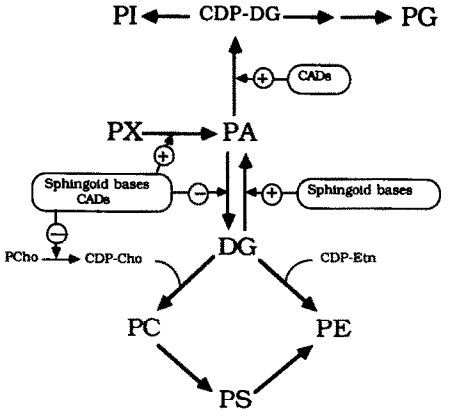

Fig. 2. Schematic presentation of major pathways of phospholipid metabolism and their modulation by CADs and by sphingoid bases. Abbreviations: CDP-Cho, CDP-choline; CDP-Etn, CDP-ethanolamine; DG, diacylglycerol; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; and PX, any phospholipid substrate of phospholipase D.

oxidative phosphorylation is inhibited [20]. Another enzyme which is affected similarly by CADs and sphingoid bases is Na^+,K^+-ATPase [21–23]. Altogether we have identified seven reactions in which there is close parallelism between the effects of representative CADs and of sphingoid bases (Table 1).

Sphingoid bases as endogenous cationic amphiphilic "drugs"

Noting the structural and functional similarities between sphingoid bases and CADs, we propose that the naturally occurring sphingoid bases may be considered as endogenous cationic amphiphilic "drugs". In other words, it is suggested that identified targets for CAD action could serve as targets of endogenous sphingoid bases, generated *in vivo* under various physiological and/or pathophysiological conditions. This proposition has several possible and potentially important implications. First, it may be predicted that further comparative studies will reveal additional pathways which are affected similarly by compounds belonging to these two groups. For example, it will be interesting to test whether any sphingoid base would inhibit dopamine receptor binding as some CADs do [24]. On the other hand, certain CADs might be predicted to increase the affinity and the tyrosine-kinase activity of the epidermal growth factor (EGF) receptor, as some sphingoid bases do [25]. Thus, by stimulating directed research, the present proposition is likely to expand the range of biochemical actions which these compounds are known to affect.

While heretofore we emphasized the gross similarities between CADs and sphingoid bases, it should be noted that there is a large degree of structural specificity in the action of individual CADs and sphingoid bases on various processes. This has obviously been very well established for CADs, where differences in *in vitro* effectiveness parallel their very different clinical applications. However, recent detailed examination of the inhibition of protein kinase C and of the EGF receptor kinase by sphingoid bases has revealed a high degree of structural specificity in their action [25–27]. Igarashi *et al.* have reported recently that synthetic *N,N*-dimethyl-D-erythrosphingenine acts as a stereospecific activator of the EGF receptor tyrosine kinase; in contrast *N,N*-dimethyl-L-erythrosphingenine, *N*-monomethyl-D-erythrosphingenine, D-erythrosphingenine, *N*-acetyl-D-erythrosphingenine, and several lyso-sphingolipids are all inactive [25]. Igarashi *et al.* have also reported that *N,N*-dimethyl-D-erythrosphingenine is a superior inhibitor of protein kinase C as compared to D-erythrosphingenine; *N,N*-dimethyl-D-erythrosphingenine stimulated v-src and c-src kinase activities, whereas D-erythrosphingenine inhibited them [27]. These studies demonstrate that sphingoid bases exhibit a high degree of specificity in their modulatory effects on various protein kinases. It might be predicted, therefore, that a similar degree of specificity will be evinced by sphingoid bases acting on other targets.

A second, more remote implication of the present proposal concerns the possible clinical usefulness of sphingoid bases. Since CADs are employed clinically in treating a large variety of disorders, the possibility arises that exogenously administered sphingoid bases (and their synthetic derivatives) may have similar therapeutic effects. For obvious reasons, as yet nothing is known on the pharmacokinetics of

Table 1. Common biochemical effects of CADs and sphingoid bases.

Enzyme or process	Effect	Reference	
		CADs	Sphingoid bases
Phosphatidic acid phosphohydrolase	Inhibition	[10, 11]	[8]
CTP:phosphocholine cytidylyltransferase	Inhibition	[12]	[13]
Phospholipase D	Activation	Unpublished*	[7]
Protein kinase C	Inhibition	[16]	[4, 5]
Ca^{2+}/Calmodulin-dependent enzymes	Inhibition	[17]	[18]
Na^+,K^+-ATPase	Inhibition	[21, 22]	[23]
Oxidative phosphorylation	Uncoupling	[19]	[20]

* Lavie Y and Liscovitch M, unpublished results.

exogenous sphingoid bases administered *in vivo* and how it may be affected by various structural modifications. Moreover, we are not aware of any studies in which pharmacological responses to sphingoid bases were examined *in vivo*. Yet, as discussed above, novel drugs with sphingoid-like structure are likely to share with traditional CADs a similar spectrum of therapeutic activities.

An intriguing aspect of the CAD-like function of sphingoid bases stems from the fact that they are natural cellular constituents. Normally, tissue levels of free sphingoid bases and lyso-sphingolipids are very low [2, 3, 6]. Hence, therapeutic strategies might be considered that involve modulation of sphingolipid metabolism and that would lead to elevated levels of endogenous free sphingoid bases. Drugs that target enzymes involved in either sphingolipid hydrolysis or sphingoid base utilization may thus have unexpected clinical benefits. On the other hand, the present hypothesis should encourage a closer look at the changes in free sphingoid bases that may occur in the aftermath of CNS insults such as ischemia, hypoglycemia, hypoxia, generalized seizures and electroconvulsive shock treatment (ECT). Can the therapeutic efficacy of ECT in the treatment of depression be due to increased levels of endogenous free sphingoid bases, mimicking the action of cationic amphiphilic antidepressants?

Concluding remarks

Sphingoid bases are natural cationic amphiphilic compounds that mimic many of the biochemical actions of CADs. In view of the structural and functional similarities that exist between sphingoid bases and CADs, we proposed that sphingoid bases may act as the endogenous counterparts of cationic amphiphilic drugs. This proposal should, hopefully, stimulate research exploring further the biochemical processes which are affected by cationic amphiphilic compounds, both naturally occurring and synthetic. Yet, given the functional similarity of sphingoid bases and CADs, and the wide utilization of the latter in clinical medicine, attempts to develop novel, sphingoid base-like drugs seem warranted. Finally, the activities of sphingoid bases as biological response modifiers, amply demonstrated in recent years, certainly stress the need to understand the metabolism of endogenous sphingoid bases and their role(s) in cell physiology and pathology.

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