Distribution of sphingosine 1-phosphate, a bioactive sphingolipid, in rat tissues

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Abstract Although a growing number of reports on the bioactivities of sphingosine 1-phosphate (Sph-1-P) in various systems have been appearing recently, current evidence for the involvement of Sph-1-P in signal transduction or cellular function(s) consists largely of data on cellular and biochemical effects of exogenous Sph-1-P. In this study, we measured Sph-1-P contents in various rat tissues. When the amount of this sphingoid base was measured by its *N*-acetylation into [^{3}H]C_2-ceramide 1-phosphate with [^{3}H]acetic anhydride, we could detect Sph-1-P in all rat tissues examined. We also got unexpected findings that Sph-1-P exists most abundantly in testis = intestine > spleen (in the order of Sph-1-P abundance when its amounts were adjusted to the phospholipid levels). Our new data on Sph-1-P distribution in rat tissues may imply novel bioactivities for Sph-1-P.

Key words: Bioactivity; Sphingosine 1-phosphate

1. Introduction

The phosphorylated sphingoid base sphingosine 1-phosphate (Sph-1-P) is the initial product of the catabolism of sphingosine (Sph) by Sph kinase and has recently been added to the list of bioactive sphingolipids [1,2]. Sph-1-P has been shown to be involved in a variety of cellular functions, including the stimulation of fibroblast growth [3,4], regulation of cell motility [5,6], platelet activation [7], activation of muscarinic K^+ currents [8], mediation of FcεRI antigen receptor signaling [9], neurite retraction [10], and the suppression of ceramide-mediated apoptosis [11]. It is now widely accepted that Sph-1-P itself has physiologic functions in addition to its role as a metabolite of Sph.

Although the physiological roles of Sph-1-P are strongly suggested, current evidence for the involvement of Sph-1-P in signal transduction or cellular function(s) consists largely of data on cellular and biochemical effects of exogenous Sph-1-P. Few studies have reported the presence and quantitative changes of Sph-1-P in cells or tissues under physiological conditions. To obtain clear evidence implicating endogenous Sph-1-P in signal transduction and to assess its physiological and pathophysiological functions, we recently developed a facile and sensitive method for quantification of Sph-1-P by its acylation into *N*-[^{3}H]acetylated Sph-1-P ([^{3}H]C_2-ceramide 1-phosphate) with [^{3}H]acetic anhydride [12]. Using this assay method, blood platelets were found to contain high levels of Sph-1-P [7,12], which is in agreement with the facts that platelets possess high Sph kinase activity and lack Sph-1-P lyase activity [13–15]. In this study, we measured Sph-1-P contents in various rat tissues.

2. Materials and methods

2.1. Sph-1-P extraction from rat tissues

The following tissues were obtained from Wistar rats (male, if not indicated): spleen, testes, muscle, kidney, heart, lung, liver, brain, intestine, and ovaries. The tissues were drained of blood, rinsed in cold physiological salt solution, and cut into smaller pieces. Weighed tissue samples were homogenized using a Brinkman Homogenizer in 6 ml of ice-cold chloroform/methanol (1:2). This was followed by thorough mixing and sonication for 30 min. After the addition of 4 ml of chloroform, 4 ml of 1 M KCl, and 150 μl of 7 N NH_4OH, the samples were thoroughly mixed and the phases separated by centrifugation. The lower chloroform phases were saved for the phospholipid determination. The alkaline upper phases were transferred to new tubes, into which 6 ml of chloroform and 300 μl of concentrated HCl were added. The samples were thoroughly mixed and the phases separated by centrifugation. The resultant upper phases were discarded and the lower chloroform phases were transferred to new tubes and evaporated to dryness under a flow of nitrogen. This was followed by a rinse of chloroform, evaporation to dryness, a rinse of ethanol, and a final evaporation. The samples were then assayed for Sph-1-P as described below.

2.2. Quantitative measurement of Sph-1-P

Sph-1-P was quantitatively measured by its *N*-acylation with [^{3}H]acetic anhydride into *N*-[^{3}H]acetylated Sph-1-P ([^{3}H]C_2-ceramide 1-phosphate) as described previously [12]. The amounts of Sph-1-P extracted were calculated by extrapolation from Sph-1-P standards run through the same procedures.

2.3. Phospholipid quantitation

The lower chloroform phases from the first extraction of the tissue samples were assayed for phosphorus content. This was performed by the method of Chen et al. [16].

3. Results and discussion

When a mass-spectrometric approach was applied to analyze Sph-1-P, its presence in rat tissues was demonstrated in the abundance order of brain > kidney > liver (adjusted to the wet tissue weights) [17]. Our results with the use of a newly-developed assay method were consistent with this rough estimation reported. That is, Sph-1-P exists in those tissues in the order of brain > kidney > liver, when the amount of this sphingoid base was measured by its *N*-acylation into [^{3}H]C_2-ceramide 1-phosphate with [^{3}H]acetic anhydride and

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adjusted to the wet tissue weights (Fig. 1). However, we also got unexpected findings that Sph-1-P exists more abundantly in testis, intestine, and spleen (Fig. 1). When Sph-1-P amounts were adjusted to the phospholipid levels, its contents in these three tissues were much higher than that in brain (in the order of testis = intestine > spleen) (Fig. 2).

One possible explanation for Sph-1-P abundance in spleen is that this organ is a reservoir for blood cells, including platelets, which are highly abundant in Sph-1-P. However, at the present stage, we do not have a clear answer to the question why Sph-1-P is abundantly present in testis. We also examined Sph-1-P content in the ovary of female Wistar rats. The values (≈25 nmol Sph-1-P/wet tissue weight in grams or 0.1 mol% Sph-1-P/phospholipid) were much lower than those in testis. Accordingly, testis, as a reproductive gland, is specifically rich in Sph-1-P. We have no good explanation for Sph-1-P abundance in intestine, either. The most important information, we believe, is Sph-1-P localization in these tissues. For this purpose, we are aiming generation of anti-Sph-1-P antibody.

We could detect Sph-1-P in all rat tissues examined (Figs. 1 and 2). Among those, Sph-1-P content was extremely low in liver. This may be related to the fact that the lyase activity for Sph-1-P degradation is very high in this organ [18]. On the contrary, we previously found that in human platelets lacking the lyase, Sph-1-P content is high and that accumulated Sph-1-P is released upon stimulation, resulting the accelation of thrombosis [7].

A growing number of reports on the bioactivities of Sph-1-P in various systems have been appearing recently. The involved cells or tissues are hematopoietic cells [7,9,11], melanoma cells [5], neuronal cells [10], fibroblasts [3,4], smooth muscle cells [6], and atrial myocytes [8]. However, in our knowledge, this is the first report to have measured the tissue distribution of Sph-1-P thoroughly. Our new data, the high Sph-1-P content in intestine and testis, might deserve special attention to get insights into novel bioactivities for Sph-1-P in these tissues or others.

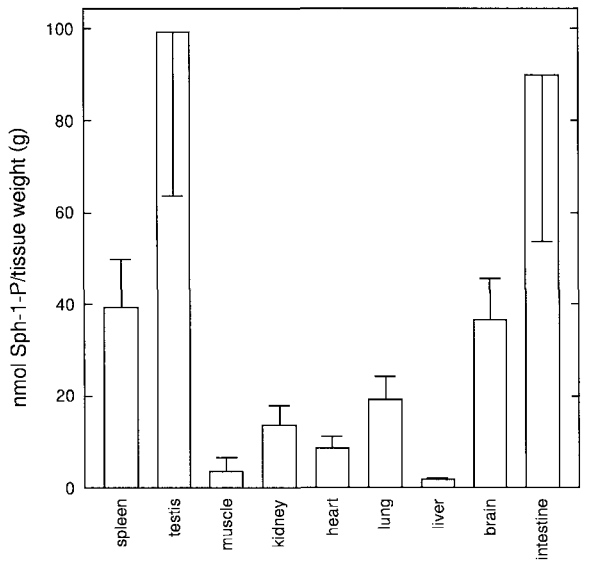

Fig. 1. Sph-1-P contents (nmol/tissue weight) in various rat tissues. Sph-1-P extracted from various rat tissues was measured and adjusted to the wet tissue weights. Values are the means ± SD ($n = 3$ for heart and $n = 4$ for all other organs).

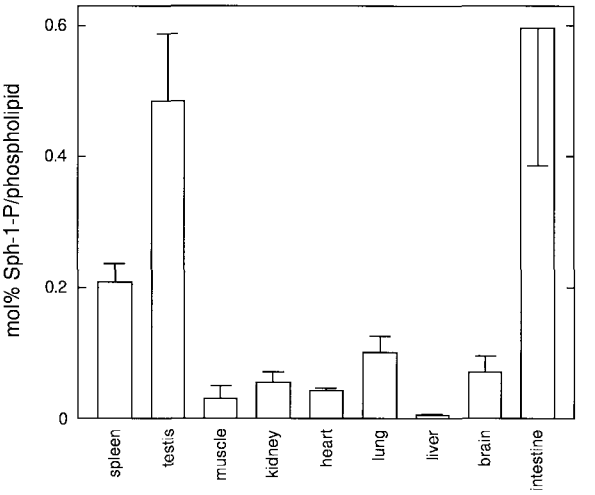

Fig. 2. Sph-1-P contents (mol%/phospholipid) in various rat tissues. Sph-1-P extracted from various rat tissues was measured and adjusted to the phospholipid levels. Values are means ± SD ($n = 3$ for heart and $n = 4$ for all other organs).

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