WALAILAK JOURNAL
http://wjst.wu.ac.th MiniReview

A Review of the Role of Sphingosine-1-Phosphate in the Brain: An Important Mediator Implicated in the Central Nervous System

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Received: 20 May 2013, Revised: 7 September 2013, Accepted: 21 November 2013

Abstract

Sphingosine-1-phosphate (S1P) is a bioactive lipid that signals through a family of G protein-coupled receptors, consisting of 5 members, termed S1P1, S1P2, S1P3, S1P4 and S1P5. Activation of S1P results in the regulation of several cellular functions, including cell proliferation, process retraction, cell survival and migration. Deregulation of S1P receptor expression and/or the S1P signaling pathway may play an important role in the development in the treatment of neurological disorders. The aim of the present review is to summarize the S1P signaling pathway and S1P receptor expression in the cellular components of human and rodent brains. The published information will be helpful to delineate the important mechanisms in the development of neurological disorders such as Alzheimer's disease, multiple sclerosis, and traumatic brain injury, and serve as a guideline for the designing of further studies investigating S1P involvement in the brain.

Keywords: Sphingosine-1-phosphate, brain, neurons, glial cells, brain endothelial cells

Introduction

Sphingosine-1-phosphate (S1P) is a lipid mediator that regulates a variety of cellular functions, including cellular proliferation, migration, and survival in several tissues and cell types [1,2]. It is produced by the phosphorylation of sphingosine by sphingosine kinases (Sphk) and inactivated by S1P phosphatases or S1P lyase (S1PL). There are 2 isozymes of Sphk, namely Sphk1, which stimulates growth and survival, and Sphk2, which promotes apoptosis [3,4]. S1P can act as a second messenger intracellularly through 5 G-protein coupled receptors, including S1P1, S1P2 S1P3, S1P4, and S1P5, which are differentially distributed and expressed in various cell types [5,6]. The human brain demonstrates high levels of expression of several S1P receptors, such as S1P1 and S1P5, and also differentially regulates relative expression of these receptors by downregulating them, leading to regulation of cellular processes [7]. In the past, S1P was thought to be mainly derived from platelets and endothelial cells; it was believed that S1P stabilized when bound to serum albumin and was released from platelets at sites of tissue injury and inflammation to promote angiogenesis [8,9]. Very recently, red blood cells, rather than platelets, have been identified as the main source of plasma S1P [10]. The S1P levels found in lymph appear to be regulated by lymphatic endothelium [11]. S1P receptors are thought to play a number of roles in brain cell function, including astrocyte proliferation and migration [12-14], oligodendrocyte differentiation and cell survival [15,16], and neurite outgrowth and neurogenesis [17]. The concentration of S1P is increased during pathological situations, such as ischaemia, traumatic injury, and disruption of the blood brain barrier (BBB) [18].

The aim of the present paper is to summarize the knowledge *in vitro* and *in vivo* studies that have investigated the mechanism of the roles of S1P and its functions in the brain cellular compartments, including neurons, glial cells, and brain endothelial cells (**Table 1**). An understanding of the mechanism of the S1P/S1P receptor signaling pathway in the brain will serve as a guideline for development of novel therapeutic treatments for neurological disorders in the future.

Expression of S1P in the central nervous system (CNS)

S1P receptors are widely localized in the central nervous system (CNS), dependent on region-specific distribution. In postnatal mouse brain at day 21, expression of the S1P5 receptor is distributed in the white matter tracts of the brain, including the corpus callosum, the fimbria, the anterior commissure, bundles of white matter running through the striatum, and the deep white matter of the cerebellum [15]. The mRNA for S1P5 is predominantly expressed in the white matter tracts of the brain, including the cerebellum, corpus callosum, and spinal cord [15,19,20]. This finding is similar to an evidence in adult rat brain, which demonstrated prominent S1P5 mRNA in the white matter [21]. It has been suggested that expression of S1P5 mRNA is localized in the mature oligodendrocytes and/or fibrous astrocytes [15,21]. A recent study investigating paraffin-embedded sections of the adult human CNS by immunohistochemistry demonstrated that fine granular staining of S1P1 receptor is stronger in the gray matter (cerebral cortex, subcortical gray matter, cerebellar cortex, brain stem nuclei, and spinal cord gray matter) than in the white matter [22]. Sphk2 mRNA and Sphk activity showed a heterogeneous distribution, especially in the cortex region, indicating that this enzyme may be involved in the interaction between different cell types in the normal adult brain during development or in pathological conditions [23]. In the CNS, S1P is secreted in both autocrine and paracrine manners, and is believed to mediate cellular actions via 3 mechanisms. First, the relative levels of S1P and ceramide can alter the microlipid environment in glial and neuronal cells. Thus, these molecules could alter the myelin forming capacity of oligodendrocytes and the synaptic function of neurons. Second, S1P is thought to act as an intracellular messenger; however, there is little known about the intracellular and/or intranuclear targets of S1P in brain cellular components. It is known that intracellular S1P can release calcium from internal stores independently of inositol triphosphate (IP_3) [24]. Third, S1P mediates its effects via activation of cell-surface S1P receptors, which are ubiquitously expressed in the CNS. All S1P receptors, except S1P4, are found to be differentially expressed on glial cells and neurons of the CNS. S1P4 receptor is thought to be restricted to the hematopoietic system and not expressed in the CNS [12]. Oligodendrocytes express mainly S1P1 and S1P5 receptors [15,16]. Neurons and astrocytes express mainly S1P1 and S1P3 mRNA, with lower level of S1P2 and no or negligible amount of S1P5 [13,14,25]. Microglial cells express S1P1 mRNA with moderate levels of S1P2 and S1P3 and little or no S1P5 [26]. Furthermore, the level of protein expression is a partial indicator of role and activity, where the nature of G protein coupling and sensitivity to activators are also important factors, even if the receptor is apparently expressed at low levels.

S1P signaling pathway and its functions in neurons

There is an evidence that S1P plays an important role in the adult and developing CNS. The S1P2 receptor is expressed in young differentiating neuronal cell bodies and axons, and is found in growing axons as well as neuronal cell bodies during the early stages of differentiation [17]. In neural progenitor cells, S1P plays a pleiotropic role in neurogenesis via the S1P receptors and in activation of intracellular transductions involving phosphorylation of extracellular signal regulated kinase (ERK) and Rho kinase [25]. Several mRNA transcripts of S1P1, S1P2, S1P3, and S1P 5 receptors were found to be expressed in the neural progenitor cells [25]. In addition, phosphorylation of ERK was emphatically induced at 10 min by adding S1P in a concentration-dependent manner, whereas the total ERK level was unaltered. In addition, the S1P1 receptor was found in the Purkinje cells of the cerebellum [27]. However, few studies of S1P in the neuronal cells have been conducted. Further studies will be required in this research area.

S1P signaling pathway and its functions in oligodendrocytes

Of the 5 S1P receptors, S1P5 is involved in regulating oligodendrocyte function. S1P5 receptors are restricted to oligodendrocytes and are preferentially expressed in oligodendrocyte lineage cells (OPC), including oligodendrocyte progenitor cells and immature/mature oligodendrocytes [15,28]. The S1P activation of S1P5 receptors can modulate several functional pathways involved in cell proliferation, process retraction, cell survival, and migration, depending on the developmental stages of the cells. In the immature oligodendrocytes, S1P5 activation mediated by S1P can induce a retraction process through the Rho kinase/collapsing response-mediated protein signaling pathways; however this retraction process is

no longer observed at later development stages [15]. In mature oligodendrocytes, activation of S1P can promote cell survival via the induction of Akt phosphorylation, which is mainly prevented by incubation of pertussis toxin (PTX) [15]. A previous study investigating the role of Sphk1 in oligodendrocytes demonstrated that neurptrophin-3 (NT-3), a stimulator of cAMP-response element binding protein (CREB) phosphorylation, can induce translocation of Sphk1 from the cytoplasm to the plasma membrane of oligodendrocytes [16], suggesting that Sphk1 appears to play a crucial role in the stimulation of oligodendrocyte progenitor survival by NT-3. In addition, modulation of S1P by a platelet-derived growth factor (PDGF) leads to the downregulation of S1P5 receptors and upregulation of S1P1 receptors in OPC, suggesting both receptors may serve different functions during oligodendroglial development and remyelination [29]. A recent study proposed that binding of S1P to S1P5 receptors results in reduction of integrin-mediated OPC migration, together with the notion that $G\alpha_i$-linked S1P1 and S1P3 are chemoattractant receptors, whereas $G\alpha_{12/13}$-linked S1P2 and S1P5 are chemorepellant receptors, suggesting that S1P selectively employs the S1P5 receptor signaling pathway to inhibit OPC migration [30]. By comparison with other cellular components in the brain, the mRNA levels for S1P5 are undetectable or low in neurons [25] and astrocytes [31]. Interestingly, the release of glutamate from neurons has been proposed to promote the release of S1P from oligodendrocytes, allowing for neuron-oligodendrocyte communication [30]. Although many previous studies have demonstrated the expression of S1P receptors and their functions, few have proposed the S1P-mediated intracellular signaling pathway in oligodendrocytes.

S1P signaling pathway and its functions in astrocytes

However, recent studies have indicated that astrocytes play a role in neuronal and oligodendrocyte protection [32]. The communication of endothelial cells with the perivascular endfeet of astrocytes appears to be important in the regulation of the BBB [33]. A recent study using the double immunohistochemistry method revealed that in normal human brain tissue, strong positive staining of S1P1 receptors in the membrane of astrocyte foot processes of glia limitans and astrocytes with radial cytoplasm [22]. In contrast to evidence in neurological disorders, hypertrophic astrocytes with strong expressions of glial fibrillary acidic protein demonstrated significantly decreased expressions of S1P1 receptors, which are modulated by inflammatory cytokines, including interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α) [34]. However, strong expressions of S1P1 were found in the astrocytes forming fibrillary gliosis, suggesting the level of S1P1 expression may change during the processes after brain damage [22]. Ultrastructural observation demonstrated that the S1P1 receptor is preferentially localized in the protoplasmic astrocyte processes surrounding neurons, suggesting the S1P/S1P1 signaling pathway may be involved in the formation of the process and maintenance of astrocyte-neuron contact [22]. There is evidence that the mRNA and protein expression of the S1P3 receptor and SphK1 increased after treatment of lipopolysacharide (LPS) as the proinflammatory cytokine stimulus, leading to elevated phosphorylation of ERK1/2 and subsequent secretion of neuroprotective chemokines, such as chemokine (C-X-C motif) ligand 1 (CXCL1) [35]. The S1P action appears to be a part of an autocrine/paracrine cascade stimulated with the basic fibroblast growth factor (bFGF), which may be associated with physiopathological glial proliferation. The bFGF can stimulate S1P release from cerebellar astrocytes in an extracellular setting, in turn stimulating cell proliferation; this process is antagonized by ceremide [36]. Expression of S1P3 and S1P1 and some S1P2 mRNA has been found mainly in rat cortical and striatal astrocytes [12,31,37,38]. Several *in vivo* and *in vitro* studies have proposed that the activation of S1P receptors leads to astrogliosis and proliferation of astrocytes [14,31,37]. In rat cortical astrocytes, S1P can also trigger the pathway for regulating production of glial cell line derived neurotrophic factor (GDNF) and cell growth, suggesting that several mechanisms mediate this process involved in protein C, Gi/o protein, and phosphatidylinositol 3-kinase [14]. In rat striatal astrocytes, the activation of S1P1 and S1P3 receptors stimulated early transduction signals of ERK phosphorylation, inhibition of cAMP production, and stimulation of phosphoinositide hydrolysis with increased Ca^{2+}, leading to the induction of astrocyte proliferation [37]. In vitro experiments reported that S1P increases cell numbers and stimulates the proliferation of astrocytes in a manner that involves ERK phosphorylation [14,31,37].

S1P signaling pathway and its functions in microglia

Microglia, known to be resident macrophages of the CNS, normally respond to neuronal damage and remove damaged cells by phagocytosis, and are involved in CNS inflammatory response [39]. S1P acts as an upstream factor, which induces the production of proinflammatory cytokines and neurotoxic substances, such as nitric oxide (NO) [40]. A recent report suggested that suppression of Sphk1 activity reducing S1P may be considered a possible therapeutic mode for the control of production of proinflammatory cytokines and NO, contributing to neuroinflammation [40]. In addition, it has been reported that decreased levels of proinflammatory cytokines, such as TNF-α and interleukin-1 beta (IL-1β), are associated with the inhibition of Sphk1 [41]. Further research focusing on the modulation of Sphk1 and S1P in microglia will serve as a guideline for the development of therapeutic drugs for the control of neuroinflammation in neurodegenerative diseases.

S1P signaling pathway and function in brain endothelial cells

Endothelial cells of the BBB express S1P receptors and Sphk1, and also release S1P. The S1P receptor system regulates the endothelial cells of the BBB by various mechanisms. First, S1P1 receptors, on endothelial cells, when activated by Fingolimod (FTY720), directly promote adherent junction assembly and tighten the endothelial barrier [42]. S1P1, via a G_i/Akt/Rac pathway, has been shown to play a role in the activation and redistribution of the tight-junction associated protein Zonula Occludens-1 (ZO-1), which enhances endothelial migration and barrier integrity [43,44]. Second, S1P1 and S1P3 receptors regulate activity of the P-glycoprotein, which is expressed in brain capillaries. This regulation of P-glycoprotein by S1P receptors may contribute to the permeability of endothelial cells and the BBB [45]. Finally, as discussed above, S1P receptors play an important role in the communication between astrocytes and endothelial cells, where astrocytes are known to regulate the BBB [32,33]. It has been reported that S1P also protects endothelial cells from apoptosis through activation of phosphatidylinositol 3-kinase/Akt/eNOS via S1P1 and S1P3 receptors [46].

Table 1 The roles of S1P and its function in brain cellular compartments.

Cell type	Functions of S1P	Reference
Neurons	Neurogenesis of neural progenitor cells via the phosphorylation of ERK and Rho kinase	[25]
Oligodendrocytes	Induces retraction process through the Rho kinase/collapsing response-mediated protein signaling pathway in immature oligodendrocytes	[15]
	Promotes cell survival via the induction of Akt phosphorylation in mature oligodendrocytes	[15]
	Oligodendroglial development and remyelination	[29]
	Reduction of integrin-mediated OPC migration	[30]
	Neuron-oligodendrocyte communication via the release of glutamate from neurons	[30]
Astrocytes	Formation of the process and maintenance of astrocyte-neuron contact	[22]
	Astrogliosis and proliferation via ERK phosphorylation	[14,31,37]
	Regulates production of glial cells line derived neurotrophic factor (GDNF) and cell growth involved in protein C, Gi/o protein, and phosphatidylinosital 3-kinase	[14]
Microglia	Induces the production of proinflammatory cytokines and neurotoxic substances such as NO	[40]
	Decreases levels of proinflammatory cytokines by inhibition of Sphk1	[41]
Brain endothelial cells	Activation and redistribution of the tight-junction associated protein Zonula Occludens-1 (ZO-1), enhancing endothelial migration and barrier integrity	[43,44]
	Regulates activity of P-glycoprotein	[45]
	Communication between astrocytes and endothelial cells	[32,33]
	Protects from apoptosis through activation of phosphatidylinositol 3-kinase/Akt/eNOS	[46]

Conclusion and recommendations

Previous studies of both *in vivo* and *in vitro* experiments have demonstrated that deregulation of S1P receptor expression and/or the S1P signaling pathway appear to play an important role in the central nervous system. Activation of S1P receptors by S1P is involved in the regulation of the cellular process proliferation, process retraction, migration, and survival in a variety of brain cellular compartments. The expression of S1P receptors is differentially distributed and expressed on various cell types. In addition, the binding of S1P to S1P receptors can trigger intracellular signaling pathway cascades, depending on the regulation of S1P receptor subtypes. While several previous studies have investigated the S1P/S1P signaling pathway in the brain, S1P-mediated intracellular signaling and dysregulation of S1P/S1P receptors and Sphk in the brain remain poorly understood. It is essential to focus on this aspect of S1P, since it may be implicated in neurological disorders. In future works, with respect to immunohistochemistry staining, the double labeling staining method providing a cell surface marker will be beneficial in identifying cell types and counting the number of target cells showing positive staining. For example, cell surface markers in CNS include glial fibrillary acidic protein (GFAP) for astrocytes, neuron-specific nuclear protein (NeuN) for neurons, and myelin cyclic nucleotide phosphodiesterase (CNPase) for oligodendrocytes. In addition, the significant role of S1P/S1P receptor signaling in the CNS should be determined by using effective and accurate methods to gain more knowledge about and to

understand the role of S1P/S1P receptor signaling, including Western blotting analysis and real time polymerase chain reaction (real-time PCR). Alterations of S1P and S1P receptors assessed at the quantitative level cannot directly predict corresponding alterations in the level of functional proteins. Increased or decreased S1P and S1P receptors as determined by the immunohistochemistry method do not reflect the downstream processes of protein synthesis, modification, folding, stability, and appropriate location that comprise the criteria for a functional gene product. For better understanding of the S1P/ S1P receptor signaling pathway, efficient molecular techniques, such as Western blotting, real-time PCR, gene knockout, DNA microarray, and proteomics will be required to verify biological functions for evidence of the alteration of the S1P/ S1P receptor signaling pathway in the normal brain and during pathological conditions. Finally, measurement of S1P receptors expression may be useful as a prognostic indicator. Understanding the function and regulation of the S1P/S1P receptor pathway could aid in the development of powerful therapeutic drugs for neurological disorders.

Acknowledgements

I would like to thank Assistant Professor Mayuree Vasinanukorn, MD, Dean of the School of Medicine, Walailak University, for her encouragement and support during the preparation of this article.

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