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COMMENTARY

Sphingosine and the transient receptor potential channel kinase(s)

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Transient receptor potential melastatin 7 (TRPM7) is a multifunctional ion channel playing crucial roles during development. TRPM7 is a member of the highly diverse TRP ion channel family. Most TRP channels are regulated by membrane phospholipids, especially phosphoinositides. In this issue of the *British Journal of Pharmacology*, Qin *et al.* describes the regulation of TRPM7 and its close homologue TRPM6 by a different kind of membrane lipid: sphingosine. The study finds that sphingosine is a potent and specific inhibitor of TRPM7 and TRPM6 channels. This commentary briefly summarizes the findings of the study, their potential significance and discusses open question and future directions.

LINKED ARTICLE
This article is a commentary on Xin Qin *et al.*, pp. 1294–1312 of this issue. To view this paper visit http://dx.doi.org/10.1111/bph.12012

Abbreviations
CDase, ceramidase; PI(4,5)P_2, phosphatidylinositol 4,5-bisphosphate; S1P, sphingosine-1-phosphate; SK, sphingosine kinases; SMase, sphingomyelinase; SPH, Sphingosine; SPT, serine palmitoyl transferase; TRP, transient receptor potential; TRPM6, transient receptor potential melastatin 6; TRPM7, transient receptor potential melastatin 7

Transient receptor potential melastatin 7 (TRPM7) is a member of the TRP ion channel family. TRPs are a highly diverse set of ion channels, they are involved in a large number of biological processes and their activation mechanisms are very diverse. In this respect, TRPM7 is a typical TRP channel, as it has been implicated in many different biological processes (Runnels, 2011). These proposed functions include cellular Mg^{2+} homeostasis, cell motility, anoxic cell death and trace metal ion transport. Despite some controversies on its exact biological role(s), TRPM7 clearly plays fundamental roles in basic cellular processes during development, since global knockout of the TRPM7 gene is early embryonic lethal in mice (Jin *et al.*, 2008). Specific ablation of TRPM7 in various lineages pointed to the importance of this channel kinase in the development of multiple organs, including the thymus (Jin *et al.*, 2008), the kidneys and dorsal root ganglia (Jin *et al.*, 2012). This is stark opposite of most TRP channel knockout mice, which usually have moderate phenotypes, either a specific non-lethal defect, or in many cases relatively subtle phenotypes. The closest homologue of TRPM7 is TRPM6. Loss of function mutation of TRPM6 causes a human disease, familial hypomagnesaemia with secondary hypocalcaemia, due to a defect in intestinal Mg^{2+} absorption (Walder *et al.*, 2002). Both TRPM7 and TRPM6 have a kinase domain in their respective C-termini. Even though the crystal structure of this kinase domain has been solved (Yamaguchi *et al.*, 2001), its exact function is still not fully elucidated.

Membrane lipids have long been considered as passive structural elements that embed ion channels and serve as insulator for ionic fluxes. In the last decade or two, it is becoming more and more appreciated that membrane lipids are more than just scaffolds; they do influence ion channel activity as ligands too. The best understood biologically active lipids that influence ion channel activity are phosphoinositides. Despite the extreme functional diversity of TRP channels, most of them share regulation by phosphoinositides (Rohacs, 2007), including TRPM7 (Runnels *et al.*, 2002) and TRPM6 (Xie *et al.*, 2011). In most cases, phosphatidylinositol 4,5-bisphosphate (PI(4,5)P_2) serves as a cofactor, required for (TRP) channel activity. PI(4,5)P_2 regulation is not specific for TRP channels; this lipid is probably a common cofactor for many, perhaps even the majority of, mammalian ion channels (Suh and Hille, 2008). Generally, cellular signals that modulate PI(4,5)P_2 levels regulate lipid sensitive ion channel activity.

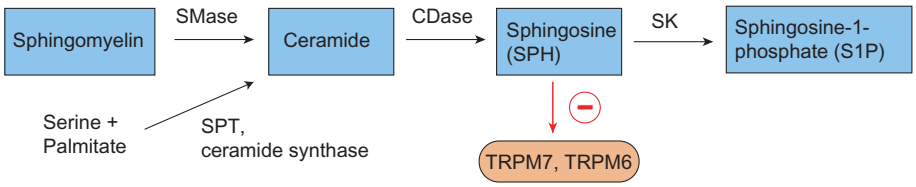

Figure 1

Sphingolipid metabolism; see text for details and abbreviations.

The article by Qin *et al.* (2012) in this issue examines the effects of another group of highly biologically active lipids, sphingolipids. The key finding of the article is that sphingosine inhibits TRPM7 and TRPM6 channel activity, by reducing open probability but not affecting single channel conductance. The inhibition was also prevalent in excised inside-out patches, where the cellular machinery is largely missing, strongly suggesting a direct effect of the compounds. The inhibition was independent of the kinase domain, since channels lacking the kinase domain were also inhibited. Sphingosine (SPH) is a bioactive lipid involved in many different biological processes (Hannun and Obeid, 2008). Sphingosine is generated from ceramide by ceramidases (CDases) (Figure 1). Ceramide on the other hand is generated from sphingomyeline by sphingomyelinase enzymes (SMases), or *de novo* from serine and palmitate by serine palmitoyl transferase (SPT) and ceramide synthase. Sphingosine is phosphorylated by sphingosine kinases (SK) to form sphingosine-1-phosphate (S1P). S1P activates GPCRs; thus, it is considered an extracellular signal. Sphingomyelin is major plasma membrane constituent, roughly accounting for 5% of the membrane lipids. The relative concentrations of these lipids decreases roughly 1 order of magnitude each step, from SM to S1P (Hannun and Obeid, 2008). Sphingolipid metabolism is very complex. There are, for example, 26 enzymes acting on ceramide. The description given here and in Figure 1 only serves as a brief orientation for those not familiar with sphingolipid metabolism.

There are several remarkable findings in the article by Qin *et al.* First, the effect of sphingosine was highly specific; neither ceramide nor S1P inhibited the channels. The experiments were performed in HEK293 cells that do not express plasma membrane receptors for S1P. Interestingly, the author's earlier work demonstrated that in cells that do express the receptor for S1P, application of this lipid inhibited TRPM7 via activation of PLC and depletion of $PI(4,5)P_2$ (Runnels *et al.*, 2002). Thus, sphingolipids exert dual control over TRPM7, both via the direct effect of SPH and the indirect effect of S1P via cell surface receptors. Second, the effect was robust and happened at low, submicromolar concentrations, which are likely to be reached physiologically. Sphingosine has been reported to have effects on other ion channels earlier, but usually micromolar concentrations were required. For example, calcium release activate Ca^{2+} (CRAC) channels are inhibited by sphingosine, with IC_{50} of 6 μM (Mathes *et al.*, 1998). Third, the effect was specific to TRPM7 and TRPM6. Other TRPM channels (TRPM2 and TRPM4) were not affected. One member of the TRPM family, TRPM3, was reported earlier to be activated by SPH in the micromolar range (EC_{50} = 12 μM) (Grimm *et al.*, 2005). Fourth, FTY720, a sphingosine derivative immunosuppressant and the first oral drug for treatment of multiple sclerosis also inhibited TRPM7. As with SPH, the addition of a phosphate group abolished the effect. The concentration of FTY720 required for inhibition of TRPM7 (IC_{50} = 0.72 μM) was over the therapeutic plasma levels used for multiple sclerosis. At higher concentrations, however, FTY720 also have anti-cancer effects, and those concentrations are sufficient to inhibit TRPM7. As developmental pathways often become re-activated in cancer, and TRPM7 was implicated in breast cancer metastasis (Middelbeek *et al.*, 2012), exploring the involvement of TRPM7 inhibition in the anti-cancer effect of FTY720 is an exciting future direction.

The effect of SPH on TRPM7 was quite robust; it essentially eliminated TRPM7 activity at concentrations that are quite likely physiologically relevant. As with any study with externally applied lipids, many questions arise, requiring further research. What is the relationship, if any, to other lipid regulators, especially $PI(4,5)P_2$? What is the molecular mechanism of inhibition? Do endogenous SPH in intact cells regulate TRPM7? Are there physiological or pathophysiological processes where the pharmacologically demonstrated effect of SPH plays a role? Given the importance of sphingolipid signalling in response to cellular stress, cell survival and apoptosis, and the involvement of TRPM7 in cellular stress responses (Aarts *et al.*, 2003), these questions are quite exciting revenues for future research.

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