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COMMENTARY

Sphingosine-1-phosphate and sphingosylphosphorylcholine: two of a kind?

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Sphingosine-1-phosphate and sphingosylphosphorylcholine are structurally related signalling molecules. Although they share some biological effects, it is debated whether this involves the same receptors. In this issue, Mathieson and Nixon report that these two lipids activate the same transcription factor but do so *via* distinct signalling pathways. Against this background, we discuss some of the potential pitfalls in studies comparing the effects of the two sphingolipids.
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Abbreviations: CREB, cAMP response element binding protein; S1P, sphingosine-1-phosphate; SPC, sphingosylphosphorylcholine

Sphingosine-1-phosphate (S1P) and sphingosylphosphorylcholine (SPC) are structurally related sphingomyelin metabolites, which in many cell types and tissues, for example in the cardiovascular system, can evoke similar responses (Alewijnse *et al*., 2004). Findings that S1P and SPC can specifically crossdesensitize each other, for example in guinea pig atrium (Bünemann *et al*., 1996) or in endothelial cells (Boguslawski *et al*., 2000), have led to the proposal that they may at least partly use the same receptors. However, in many cases S1P and SPC appear to use distinct receptors since their responses occur *via* distinct signalling pathways or are dissimilar, which includes the possibility that a cell or tissue reacts to one but not the other (van Koppen *et al*., 1996). In this issue of the Journal Mathieson and Nixon report that S1P and SPC both activate cAMP response element binding protein (CREB) in rat cerebral artery, but do so *via* distinct mitogen-activated protein kinases (Mathieson & Nixon, 2006). Based upon their findings the authors consider it unlikely that the same receptor is involved, although the possibility of ligand-directed signalling cannot be excluded based upon these data. These findings highlight the importance of careful evaluation of multiple cellular responses/pathways prior to reaching conclusions regarding the roles of S1P and SPC.

S1P and SPC are not only formed from the same precursor, that is sphingomyelin, but also can be converted into another by the exoenzyme autotaxin (Clair *et al*., 2003). Therefore, it is not always certain whether nominally added S1P or SPC indeed act in this form or have been converted. In this regard, late cellular responses such as migration or proliferation may be more susceptible to involve such conversion than early cellular responses. However, the CREB activation observed by Mathieson and Nixon cannot be explained by ligand conversion because it involves distinct signalling pathways. Another issue with regard to ligand identity is the fact that commercially available S1P and SPC not always have the same degree of purity, and that some of the associated impurities may be biologically active. For example, it was found that some SPC preparations contain contaminations, which may be responsible for the observed biological effect (Liliom *et al*., 1998).

An intriguing feature of both S1P and SPC is that they not only can act as agonists on their cognate receptors but are likely to also have intracellular effects, that is act as second messengers (Meyer zu Heringdorf *et al*., 2002). However, the identity of the intracellular targets of both sphingolipids has not been defined. Therefore, it is often difficult or even impossible to determine whether the observed effects are indeed receptor-mediated. Whereas sensitivity towards pertussis toxin, which inactivates $G_{i/o}$ G-proteins, is considered a sign of receptor-mediated S1P/SPC effects, a lack of effect of pertussis toxin is difficult to interpret since at least some of the cloned S1P receptors can also activate $G_{q/11}$ and/or $G_{12/13}$ G-proteins.

Five subtypes of cloned S1P receptors are recognized, and additional ones have been proposed. The pharmacological characterization of S1P receptors has rarely relied upon radioligand-binding studies, possibly because lipid mediators are prone to yield high nonspecific binding due to their physicochemical properties (Im, 2004). Rather, the ligand specificity of the cloned S1P receptors was mainly established by functional assays. These are complicated because virtually no existing host cell line for transfection of cloned receptors is unresponsive to sphingolipids, that is fully lacks endogenous receptors. Nevertheless, it was routinely found that the cloned receptors exhibit at least 100-fold higher potency of S1P than that of SPC for proximal cellular responses (Meyer zu Heringdorf *et al*., 2002). Therefore, SPC effects, which occur in a similar concentration range as S1P effects, are unlikely to occur *via* the cloned S1P receptors unless a rapid and complete metabolism of SPC to S1P is postulated. Rather specific SPC receptors need to be postulated, which based upon the sensitivity of many SPC effects towards pertussis toxin, are likely to belong to the family of G-protein-coupled receptors (Meyer zu Heringdorf *et al*., 2002). Indeed, at least two orphan

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receptors have been proposed to encode high-affinity SPC receptors, namely OGR1 and GPR4 (Xu, 2002). An interesting common feature of OGR1 and GPR4 is that they can signal in a pH-dependent manner and hence are also considered proton-sensing receptors (Tomura *et al*., 2005). Whether indeed one receptor can physiologically be activated by two completely distinct types of ligand, remains to be proven. Moreover, OGR1, in contrast to most SPC responses, is not sensitive to pertussis toxin (Zhu *et al*., 2001), and some investigators were unable to confirm activation of GPR4 by SPC (Tomura *et al*., 2005).

In conclusion, there is now good evidence that both S1P and SPC have receptor-mediated effects but are likely to act *via* distinct receptors or families thereof. Whereas five S1P receptor subtypes have been conclusively identified, it remains to be studied whether the proposed additional receptors indeed are physiologically activated by S1P and/or SPC. Moreover, in the pool of unassigned orphan receptors a genuine SPC receptor or one with similarly high affinity for both sphingolipids could exist. Finally, some of the described pharmacology of S1P and SPC might involve dimerization between known receptors.

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