Chemistry and Physics of Lipids 199 (2016) 74–93

Contents lists available at ScienceDirect

Chemistry and Physics of Lipids

journal homepage: www.elsevier.com/locate/chemphyslip

Active membrane cholesterol as a physiological effector

Yvonne Lange[a,*], Theodore L. Steck[b]

[a] Department of Pathology, Rush University Medical Center, 1653 W. Congress Parkway, Chicago, IL 60612, USA
[b] Department of Biochemistry and Molecular Biology, University of Chicago, 929 East 57th Street, Chicago, IL 60637, USA

ARTICLE INFO

Article history:
Received 1 December 2015
Received in revised form 4 February 2016
Accepted 8 February 2016
Available online 10 February 2016

Keywords:
Active cholesterol
Homeostasis
Receptors
Channels
Transporters

ABSTRACT

Sterols associate preferentially with plasma membrane sphingolipids and saturated phospholipids to form stoichiometric complexes. Cholesterol in molar excess of the capacity of these polar bilayer lipids has a high accessibility and fugacity; we call this fraction *active cholesterol*. This review first considers how active cholesterol serves as an upstream regulator of cellular sterol homeostasis. The mechanism appears to utilize the redistribution of active cholesterol down its diffusional gradient to the endoplasmic reticulum and mitochondria, where it binds multiple effectors and directs their feedback activity. We have also reviewed a broad literature in search of a role for active cholesterol (as opposed to bulk cholesterol or lipid domains such as rafts) in the activity of diverse membrane proteins. Several systems provide such evidence, implicating, in particular, caveolin-1, various kinds of ABC-type cholesterol transporters, solute transporters, receptors and ion channels. We suggest that this larger role for active cholesterol warrants close attention and can be tested easily.

1. Introduction

1.1. Membrane sterols

Sterols are ubiquitous and abundant in the membranes of eukaryotes, where they evolved to serve a multiplicity of functions (Bloch, 1983; Lange and Steck, 2008; Maxfield and van Meer, 2010). Free (*i.e.*, unesterified) cholesterol is maintained in many animal cells at a fairly constant level, ~0.3–0.4 mol/mol phospholipid, coordinated with their polar bilayer lipids (mostly phospholipids and sphingolipids) (Breslow and Weissman, 2010; Nohturfft and Zhang, 2009). In contrast, sterol esters are stored in droplet reservoirs, the abundance of which varies with supply and demand. Cholesterol is most plentiful and especially enriched in the plasma membrane; this pool contributes ~80% of the cell total in human fibroblasts at ~0.8 mol/mol plasma membrane phospholipid; *i.e.*, ~44 mol percent of the lipid (Lange et al., 1989, 2012, 2014). Cholesterol is also enriched in endocytic compartments by virtue of their derivation from the plasma membrane and the ingested low density lipoproteins in their lumens (Iaea and Maxfield, 2015; van Meer et al., 2008). The endocytic recycling compartment (ERC) is also enriched in sterols, perhaps reflecting the abundance of binding proteins therein (Hao et al., 2002). In contrast, the endoplasmic reticulum (ER) might contain ≤1% of cell cholesterol at ~0.05 mol/mol phospholipid (Lange et al., 1999; Radhakrishnan et al., 2008; Sokolov and Radhakrishnan, 2010). The cholesterol in other cytoplasmic membranes is also very low but has been less well quantified (van Meer et al., 2008). [Preparations of these organelles are often contaminated with plasma membrane fragments that increase their apparent sterol content (Lange et al., 1989).

1.2. Regulation of membrane sterols

The abundance of unesterified cellular cholesterol is tightly controlled; e.g., Abi-Mosleh et al. (2009),Steck and Lange (2010). Either too much or too little sterol appears to be injurious (Cui et al., 2007; Esfahani et al., 1984; Feng et al., 2003; Tabas, 2002). Regulation takes place principally in the plasma membrane, ER and mitochondria and occurs at many levels, including its biosynthesis, ingestion, storage as esters, efflux and various chemical modifications that serve specialized functions [see Sections 5 and 6 and

Abbreviations: ABCA1, ATP-binding cassette transporter A1; ABCG1, ATP-binding cassette transporter G1; CRAC, cholesterol recognition/interaction amino acid consensus; ER, endoplasmic reticulum; GABA, γ-amino butyric acid; GPCR, G protein-coupled receptors; HDL, high-density lipoprotein; HMGR, hydroxy-3-methylglutaryl-CoA reductase; HPCD, 2-hydroxypropyl-β-cyclodextrin; MBCD, methyl-β-cyclodextrin; NPC1, Niemann–Pick disease type C1; NPC1L1, Niemann–Pick C1-Like 1; NPC2, Niemann–Pick disease type C2; OSBP, oxysterol-binding protein; ORP, OSBP-related protein; PFO, *Clostridium perfringens* alpha toxin O; Scap, sterol regulatory element binding protein cleavage activating protein; StAR, steroidogenic acute regulatory protein; START, StAR related lipid transfer protein; SREBP, sterol regulatory element binding protein.

* Corresponding author.
E-mail addresses: ylange@rush.edu (Y. Lange), tsteck@bsd.uchicago.edu (T.L. Steck).

http://dx.doi.org/10.1016/j.chemphyslip.2016.02.003
0009-3084/

Ikonen, 2008; Mesmin and Maxfield, 2009; Miller and Auchus, 2011; Russell, 2003; van der Wulp et al., 2013]. The effector proteins are controlled at multiple levels, including gene expression and the synthesis, degradation, covalent modification and the regulation of the proteins. Many homeostatic activities occur on a time scale of minutes through the direct sensing of the local sterol level by these proteins.

1.3. Membrane sterol allocation

The phospholipid and sphingolipid levels in the various membranous organelles are set by the active transport of lipids between and across membrane bilayers so as to maintain their programmed non-equilibrium distribution (Contreras et al., 2010; Drin, 2014; Lagace and Ridgway, 2013). Not so with cholesterol. Rather, sterols generally appear to be apportioned according to their affinity for the various polar lipids (see Section 2.2). It has been argued that sterols can spontaneously desorb and diffuse from bilayers (Phillips, 2014). However, they are notably water-insoluble, so that their rapid intracellular movements are generally facilitated. One pathway utilizes vesicle fusion and fission to move bulk bilayer cholesterol and lumenal cargo (van Meer et al., 2008). More relevant here is intracellular sterol transport by different kinds of lipid transfer proteins (Section 9 and Iaea and Maxfield, 2015; Phillips, 2014; Prinz, 2007, 2010). Most of these proteins do not move sterols against their thermodynamic gradient nor are they coupled to metabolic energy. Rather, they facilitate passive down-hill diffusion and exchange that equilibrates sterol molecules among donor and acceptor membranes by virtue of their chemical activity (Wüstner and Solanko, 2015). Thus, these transporters can set the rates of reversible transfer processes but not their extent, even if they make specific associations with target organelle membranes (Stein and Lieb, 1986).

Nevertheless, metabolic activity can drive cholesterol away from the diffusional equilibrium that otherwise determines its steady-state distribution; for example, through its rapid synthesis, esterification, de-esterification, excretion, active transport and transformations such as steroidogenesis and hydroxylation. Some investigations have suggested that certain transfer proteins can determine the intracellular distribution of sterols even though they are not known to be coupled to an energy source; for examples, see Garbarino et al. (2012), Georgiev et al. (2011), Lavigne et al. (2010), Mesmin et al. (2011) and Prinz (2007). However, such observations have typically been made on non-steady-state processes and have often involved strong over-expression of the protein.

2. Active cholesterol mediates sterol homeostasis

Neither the facilitated diffusion of cholesterol nor its active transport accounts for how the regulatory proteins residing in one membrane (the ER and mitochondria in particular) sense the abundance of cholesterol in other loci (the plasma membrane in particular) and adjust it to the physiologically prescribed level. A parsimonious hypothesis has addressed this question through five experimentally-validated premises (Lange and Steck, 2008; Steck and Lange, 2010).

2.1. The first premise: cholesterol circulation

Cholesterol moves briskly among the cellular membranes. For example, its transfer between the plasma membrane and cytoplasmic organelles proceeds on a time scale of a few minutes, so that the entire pool of cholesterol circulates to and from the plasma membrane with a half-time of far less than an hour (Lange et al., 2014, 1999, 2002, 2009a; Wüstner and Solanko, 2015; Wustner et al., 2002, 2005). These rapid fluxes appear to be passive (Section 9). They allow multiple cellular sterol compartments to approach the same steady-state chemical activity. This enables the sterol-managing loci to monitor and regulate cellular sterol concentrations through a grand equilibrium. This rapid downhill sterol circulation would tend to discharge gradients created by its active transport, an argument against the latter as a general mechanism for its allocation.

2.2. The second premise: differential sterol affinity

It has been suggested that the heterogeneity observed in the cholesterol content of the organelles reflects, to a large degree, differences in the relative sterol affinity of their bilayer lipids (Lange and Steck, 2008; Ohvo-Rekila et al., 2002; Wüstner and Solanko, 2015). In this view of membrane biogenesis, the sterol distribution would generally reflect the relative abundance and affinity of the various polar lipids that are apportioned among and across organelle membranes by active vectorial mechanisms (Drin, 2014). Indeed, the roughly ten-fold spread in cholesterol/phospholipid mole ratios among the cell membranes parallels the free energy differences in the strengths of association of the sterol with the polar lipids resident in those bilayers (Lange et al., 2013). In particular, the large quotient of sterols in plasma membranes and their endomembrane derivatives matches the strong affinity of their saturated phospholipids and sphingolipids, while the converse is generally the case for the intracellular membranes (Lange and Steck, 2008; Lange et al., 2013; Niu and Litman, 2002; Tuller et al., 1999).

2.3. The third premise: sterol/lipid stoichiometry

Cholesterol associates with different lipids to form dynamic complexes with specific stoichiometries; typically, the sterol: phospholipid mole ratios are near 1:1 or 1:2 (Lange and Steck, 2008; Lange et al., 2013; Litz et al., 2016; McConnell and Radhakrishnan, 2003; Radhakrishnan and McConnell, 2000; Steck and Lange, 2010; Quinn, 2012). That plasma membrane bilayers contain ~0.8 mol of cholesterol per mol phospholipid is consistent with the predicted preponderance of 1:1 complexes plus a minority of lower stoichiometries in those membranes. On the other hand, the intracellular compartments appear to be well below their stoichiometric equivalence points. For example, the cholesterol:phospholipid mole ratio in the ER has been reported to be ~0.05 (Radhakrishnan et al., 2008; Sokolov and Radhakrishnan, 2010). That the intracellular membrane lipid compartments are undersaturated has been substantiated by the demonstration that their cholesterol (but not that of the plasma membrane) is increased several-fold when cells are over-loaded with exogenous sterol (Lange et al., 2014, 1999). These observations are consistent with the hypothesis that the plasma membrane provides the predominant signal for cellular cholesterol homeostasis (see Section 2.5).

2.4. The fourth premise: active cholesterol appears at a threshold

The key attribute of sterol molecules exceeding stoichiometric equivalence with their neighboring polar bilayer lipids is their high chemical activity relative to the bulk of the sterol that is held in complexes (McConnell and Radhakrishnan, 2003; Radhakrishnan and McConnell, 2000). We call this fraction *active cholesterol*. The emergence of active cholesterol is seen as an acute rise in sterol availability above a threshold. An illustration is the sharp increase in the accessibility of plasma membrane cholesterol to cholesterol oxidase at its physiologic set point (Fig. 1 and Lange et al., 1980). Similar thresholds are seen for the binding of PFO to bilayers and