Acute regulation of steroidogenesis: Endocytosis-mediated mitochondrial cholesterol supply is an imperative step that enables STAR function

Sanjeev Wasti[1], Prasanthi P. Koganti[1] and Vimal Selvaraj[1]

1. Department of Animal Science, College of Agriculture and Life Sciences, Cornell University, Ithaca, New York 14853

Recent findings that STAR functions as a cholesterol shuttle in the mitochondrial intermembrane space has brought renewed focus on upstream steps that enable colossal levels of cholesterol supply to the outer mitochondrial membrane (OMM). The current model is built on probable endoplasmic reticulum (ER) to OMM cholesterol transfer mediated by points of close apposition, termed the mitochondria associated membrane (MAM). However, two new observations from MA10 Leydig cells led us to question this model in that: (1) Induced expression of functional STAR by itself could not initiate any steroidogenesis, a finding that conflicted with the widely-accepted assumption about STAR being the rate-limiting step in steroidogenesis. (2) Blocking sterol-associated endocytosis using filipin, a macrolide antibiotic that in live cells binds plasma membrane (PM) free cholesterol, could completely abolish trophic hormone induced steroidogenesis. On this basis, we investigated the role of endocytosis in mitochondrial cholesterol supply.

Inhibition of sterol-associated endocytosis by using nystatin, another free cholesterol binding drug, provided results identical to that observed with filipin. In dose dependent responses, both filipin and nystatin treatments resulted in decreases that led to a complete cessation of progesterone biosynthesis at the high doses. This finding demonstrated that without endocytosis and delivery of PM free cholesterol, steroidogenesis is unable to proceed.

Endocytosis of cholesterol-enriched regions in the PM can occur through different modalities that include: Clathrin-mediated, Caveolae-mediated, RhoA-mediated, Endophilin-mediated, and Cdc42-mediated endocytosis. Among these, the first four types of endocytosis are dependent on dynamin, a protein that assembles as helical polymers at the neck of invaginating vesicles and induces fission to generate free endosomes. Therefore, we examined the effect of Dynasore and Dyngo 4a, two small molecules that inhibit dynamin. Our results showed significant declines to steroidogenic function approaching a complete cessation of steroidogenesis with dynamin inhibition. To confirm this pharmacology using a genetic model, we developed a doxycycline inducible lentiviral system to express a dominant negative (DN) form of dynamin 2 in MA10 cells. Induction of DN dynamin 2 significantly suppressed trophic hormone induced steroidogenesis by >80%. These findings not only confirmed that dynamin-dependent endocytosis is an essential step in channeling PM cholesterol to mitochondria, but also indicated that MAMs do not support ER to mitochondrial cholesterol transport for steroidogenesis.

As clathrin-mediated endocytosis is triggered for luteinizing hormone receptor (LHR) turnover after trophic stimulation, we evaluated whether the PM cholesterol mobilization is integrated with LHR signaling. When we used chlorpromazine, a small molecule that inhibits clathrin organization, we found significant declines (>80%) to steroidogenic capacity. This finding indicated that PM cholesterol mobilization is mechanistically coupled to trophic hormone signaling in steroidogenic cells.

These results are remarkable for three reasons: First, they indicate that all intracellular and extracellular sources need to supply cholesterol to the PM first, before transport to the mitochondria. This principle provides the means for a cell to regulate mitochondrial cholesterol supply by starting and stopping this process via a single channel. Second, they demonstrate that MAMs do not have a role in mitochondrial cholesterol supply for steroidogenesis, which is contrary to the prevailing view. Third, they reveal that STAR function cannot be rate-limiting as OMM cholesterol delivery is an

independently regulated first and essential step, that enables the second step of OMM to inner mitochondrial membrane cholesterol translocation by STAR.