Clearing the way to implantation

Commentary

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The endocrine symphony known as the reproductive life cycle consists of a complex series of hormonally driven "movements." The sole rationale for the reproductive cycle is the generation of a fertilizable ovum and, contingent upon fertilization, the establishment of implantation and of an ongoing gestation. Although fecundity rates vary among species, there can be little question as to the ongoing success of sexual reproduction.

As might be expected given their complexity, the reproductive cycle and the attendant sexual reproductive process are vulnerable to breaking down at key points. For this reason, genetic analysis of organisms with attenuated or enhanced reproductive patterns has shed considerable light on the wild-type state. Increasingly over the last two decades, the complementary strategy of reverse genetics — generally by targeted gene deletion in the mouse — has allowed researchers to examine the physiological roles of molecules presumed to act in the reproductive cycle.

The Man/GalNAc-4-SO_4 receptor and the pulsatile delivery of luteinizing hormone

In this issue of the *JCI*, Mi and associates (1) set out to elucidate the role of a specific carbohydrate-binding protein, the mannose/GalNAc-4-SO_4 receptor, in mammalian physiology in general, and in reproduction in particular. The authors attempted to generate the relevant null mutant, but the resultant *M/G4SR*$^{-/-}$ homozygotes died in utero, indicating that the Man/GalNAc-4-SO_4 receptor is indispensable for intrauterine growth and development. Faced with the lethality of the homozygous state, the authors turned their attention to the heterozygous state. They found that females carrying only a single functional allele of the receptor gene bear small litters, apparently as a result of defective implantation. This intriguing but subtle phenotype is consistent with a role for the receptor in the implantation process, presumably through clearance from the plasma of luteinizing hormone (LH), as has been hypothesized by Baenziger and his colleagues over approximately the past decade.

The Man/GalNAc-4-SO_4 receptor is an unusual protein in several respects. It was originally identified as a member of the C-type lectins, a family of calcium-dependent carbohydrate-binding proteins, and indeed, it occurs in macrophages as a monomeric cell surface protein that binds specifically to mannose-containing structures (2, 3). This form of the protein probably facilitates the clearance of bacteria and fungi that carry high-mannose structures on their cell surfaces. Remarkably, the same molecule is dimeric in hepatic endothelial cells. It is this dimeric form of the receptor that binds to Man/GalNAc-4-SO_4 (4). In the dimeric receptor, the NH_2-terminal region — rather than the C-type lectin domains that characteristically bind carbohydrate moieties — mediates binding to GalNAc-4-SO_4 (5). In this regard, the dimeric form of the receptor differs not only from previously characterized lectins, but also from the monomeric form, which employs the C-type lectin domains in binding mannose. Because of this unusual dual function, one cannot formally exclude the possibility that a deficit in the mannose-binding, monomeric form of the receptor contributes to the phenotype of animals with reduced expression of this gene. Moreover, the embryonic-lethal phenotype of *M/G4SR*$^{-/-}$ suggests that the Man/GalNAc-4-SO_4 receptor, in either its monomeric or its dimeric form, subserves some essential developmental function that is independent of the hepatic clearance of LH — a role likely assumed postnatally. Nevertheless, as discussed below, the reduced fertility observed in heterozygous female mice can plausibly be ascribed to altered LH dynamics as a consequence of reduced GalNAc-4-SO_4 receptor function.

As with several other critical reproductive hormones found in male and female mammals, LH function depends, in part, on the kinetics of hormone biosynthesis, release, and clearance. In postpubescent humans and large animals, LH is released into the plasma in a pulsatile manner. Driven by the circhoral release of the hypothalamic decapeptide gonadotropin-releasing hormone, the gonadotrophs, the anterior pituitary cells responsible for the biosynthesis and release of LH and follicle-stimulating hormone, respond with comparably paced secretion of their products. The kinetics of this process are under tight control, such that not only the amplitude and frequency of hormone release, but also the rate at which basal levels are reestablished after a pulse are relatively constant.

The pulsatile kinetics observed for LH are best explained by the periodic release of this glycopeptide hormone, followed by its rapid clearance from the bloodstream. These parameters are proposed to be critical for controlling the physiological potency of the signaling through the LH receptor. If so, it follows that events under LH control — oocyte maturation and ovulation, as well as the production of hormones that prime the uterus to support implantation — are in part dependent on LH's circulatory half-

life. Structural analyses from Baenziger's group indicate that LH is modified by GalNAc-4-SO_4, a specific and somewhat unusual glycan now known to bind the endothelial form of the Man/GalNAc-4-SO_4 receptor. They have suggested that binding of this receptor to the glycan-bearing hormone accounts for rapid clearance of LH, which is crucial to explain the pulsatile delivery of plasma LH (6). In their present article (1), this group has now explored the postulated role of the receptor in female reproduction.

The endocrine phenotype of *M/G4SR*$^{-/+}$ females

The fundamental pathology displayed by mice heterozygous for the Man/GalNAc-4-SO_4 receptor appears to be a marked reduction in the overall binding capacity of LH by the hepatic endothelium, where this receptor is most prominently expressed. Interestingly, the reduced efficiency of implantation sites could be substantially restored by providing exogenous progesterone and estrogen, hormones whose biosynthesis in the corpus luteum would otherwise be under the control of LH. For this reason, it appears that appropriate LH clearance is critical for the luteal production of sufficient amounts of progesterone and estrogen to support implantation.

The reduced expression of the Man/GalNAc-4-SO_4 receptor impairs the clearance of exogenously administered, labeled LH and would therefore be predicted to weaken the pulsatile character of LH delivery to target organs. Unfortunately, the extent of this kinetic effect is difficult to estimate. Even a mild diminution of LH clearance would be predicted to affect the pulsatility of circulating LH and to lead to an increased concentration of the hormone during the interpulse period. The most extreme form of such a disruption, the complete elimination of LH pulsatility and the continuous exposure of the ovary to elevated circulating levels of the hormone, has been modeled by overexpressing LH in transgenic mice (6). The phenotype seen in these animals includes ovarian cyst formation, ovarian tumorigenesis, and sterility, markedly different effects from those reported by Mi and associates.

The relatively mild phenotype under discussion is probably to be expected if the reduction in LH pulsatility and the consequent increase in ambient LH levels are subtle, as in the *M/G4SR*$^{-/+}$ heterozygotes. Indeed, it should be emphasized that ovulation persists in these animals and that even the implantation defect observed is relatively modest: The mean number of implanted embryos per dam was reported as 8.7 in wild-type females and 5.1 in the heterozygotes. Actual measurements of the circulating levels of LH have not been reported for the knockout mouse line studied here. Consequently, it appears likely that heterozygous deletion of *M/G4SR* is associated with a modest derangement of LH economy, the precise nature and extent of which will require additional evaluation.

Conclusions and concerns

The evidence for the presumed derangement of LH economy in the *M/G4SR*$^{-/+}$ heterozygotes rests primarily on the kinetic data indicating that the half-time of labeled LH is increased perhaps fourfold in these animals relative to wild-type controls. The finding that exogenous progesterone and estrogen help restore the efficiency of implantation in heterozygous females is consistent with the idea that this modest effect on LH economy leads to a suboptimal output of steroid hormones by the corpus luteum, but this point remains to be established experimentally. It is equally uncertain which targets benefited from the provision of exogenous estrogen and progesterone. Indeed, the exogenous steroids may well have targeted endpoints other than the uterus. For example, the administration of estrogen could have contributed directly or indirectly to upregulating the endothelial expression of the Man/GalNAc-4-SO_4 receptor.

Thus, while the work of Mi and associates (1) leaves little doubt that the Man/GalNAc-4-SO_4 receptor plays a central role in normal physiology, the significance of this receptor to LH clearance from the bloodstream — and, more broadly, to reproductive biology — should ideally be revisited in a more robust system. Much of the difficulty in interpreting the correction of the heterozygote's phenotype derives from the subtle, quantitative effect of reduced receptor expression. Conditional ablation of the receptor, or transgenesis using a construct that drives receptor expression only early in the animals' development, could bypass the embryonic defect seen in *M/G4SR*$^{-/-}$ homozygotes. Assuming that no later developmental requirement comes to light, it should be possible to generate viable, receptor-null adults in which to study the endocrine and reproductive effects of the GalNAc-4-SO_4-protein modification.

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