Sex Requires Kisses

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Summary

Hypogonadadotropic hypogonadism affects thousands of people worldwide. This study identifies Kiss1 as a ligand for the GPR54 receptor, which is involved in the proper development of reproductive organs.

Introduction

Imagine a disease that prevents you from having biological children of your own. Hypogonadotropic hypogonadism is characterized by defects in gonadal development, which results in infertility or in failure to go through puberty (1). A deficient production of follicle-stimulating hormone (FSH) and lutinizing hormone (LH) causes the disease (1). Tassigny et al., (2007) made a major discovery about the disease, showing that *Kiss1* activates GPR54, a receptor involved in causing hypogonadadotropic hypogonadism (2). In 2003, two independent research laboratories identified mutations in GPR54, a G protein-coupled receptor gene, which causes idiopathic hypogonadadotropic hypogonadism in both humans and mice (1, 3). Deleting GPR54 in mice resulted in underdeveloped reproductive organs in both female and male mice (3,4). Male mice had low levels of testosterone, while females had low levels of reproductive hormones (3,4). Previously, the studies showed that GPR54 is vital for the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus, which induces secretion of LH and FSH hormones from the anterior pituitary (5). The biological role of LH and FSH hormones is to stimulate the development of reproductive organs.

Kiss1 was initially discovered as a tumor suppressor gene (6). *Kiss1* encodes for the protein kisspeptin-54, also known as metastin due to its ability to delay metastasis (6). In addition, studies also show that exogenous delivery of kisspeptin-54 stimulates the hypothalamamic-pituitary gonadal axis and the secretion of both LH and FSH hormones in mice (6, 7). *Kiss-1* mRNA has been located in the rostral caudal area of the hypothalamus (6, 8). Also, scientists demonstrated that kisspeptins activate GnRH neurons at puberty in female and male mice (9). Until the discovery of Tassigny et al., it was not known what stimulates GPR54. Scientists hypothesized that *Kiss1* is a ligand for GPR54, since both seem to modulate the release of FSH and LH, hormones that are crucial for gonadal development. In addition, the exact physiological role of *Kiss1* was unknown.

To test the hypothesis that *Kiss1* is a true ligand for GPR54, Tassigny et al., generated *Kiss1* deletion mice. *Kiss1* knockout mice, mice that do not express the *Kiss1* gene, were created by deleting two exons in the *Kiss1* gene located on chromosome 1. Scientists inserted the *LacZ* reporter gene into one of the deleted *Kiss1* exons in order to confirm the deletion of the *Kiss1* gene. After confirming the deletion of the *Kiss1* gene, scientists began to look at the anatomy and histology of the mice. Because the physiological role of the gene was unknown, scientists looked into this subject. As expected, mutant mice only had abnormalities in the reproductive system, demonstrating that *Kiss1* does not play a major role in any other physiological processes.

One of the major observations was hypogonadism in *Kiss1* knockout mice compared to the wild-type mice. Hypogonadism is a medical term that means decreased functional activity of the gonads. The testes of *Kiss1* mutant mice were significantly smaller than wild-type testes. Also, they saw reduced development of seminal vesicles in knockout mice. Female *Kiss1* mutant mice had considerably smaller ovaries and uteri when compared to the wild-type mice.

In addition, the results showed that both female and male mutant mice did not progress through pubertal sexual maturation. In normal mice, pubertal sexual maturation is achieved by proper development of sex glands in males and vaginal opening in females. *Kiss1* mutant males had poorly developed secondary sex glands. Females' vaginas failed to open at pubertal age, and the female mice had a disrupted estrous cycle. Furthermore, histological studies of mutant mice showed abnormal development of the ovaries and the uterus. The ovaries of the *Kiss1*-/-mice did not have late-stage antral follicles or corpora lutea. In addition, mutant ovaries had a large number of atretic follicles when compared to wild-type. The uteri of the mutant mice resembled uteri of animals before puberty, an indicator that *Kiss1*-/-female mice did not go through puberty. Similarly, histological studies showed that *Kiss1*-/-male mice went through defective spermatogenesis with a deficiency of spermatozoa in the majority of the seminiferous tubules, demonstrating that male mutant mice failed to go through puberty as well.

Scientists assessed the hormonal levels in the mutant mice in order to understand the mechanisms underlying the underdevelopment of the reproductive organs. Typically, male mice exhibit high levels of testosterone, and female mice have cyclic fluctuations in 17-β-estradiol levels. The endocrine profiles showed decreased levels of sex hormones in comparisons to wild-type mice. *Kiss1*-/-male mice showed low levels of testosterone in comparisons to wild-type mice. Wild-type mice show cyclic fluctuations of 17-β-estradiol levels, while *Kiss1*-/-female mice showed no fluctuations. In addition, female mice demonstrated significantly lower levels of FSH and LH compared to wild-type mice.

One possibility for the lack of development of reproductive organs is the failure of the GnRH neurons to migrate. In order to eliminate the possibility that the lack of gonadal development is due to abnormal migration of the GnRH neurons, Tassigny et al., studied the neurons in the hypothalamus of the mutant mice. Tassigny et al., effectively used immunohistochemistry to show that GnRH neurons migrated appropriately in the hypothalamus of both *Kiss1* and wild-type mice. In addition, GnRH levels in the hypothalamic neurons showed no significant difference between the mutant and wild-type mice. Finally, in order to confirm that the hypothalamic-pituitary axes were functional, exogenous kisspeptin-10 was injected into mutant mice. After injection, LH levels in mutant female mice were significantly higher than the levels in mice that did not receive an injection. These results confirmed the proper function of the hypothalamic-pituitary axis. Most importantly, the rescue experiment confirmed that kisspeptin-10 is the only true ligand for the GPR54 receptor.

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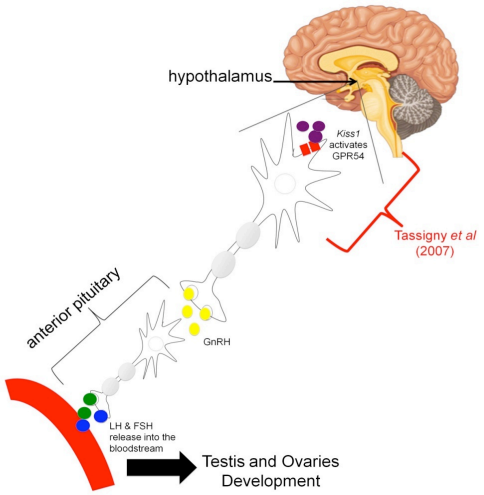

Figure 1: *Kiss1* activates GPR54 receptor in the hypothalamus
Tassigny et al., (2007) showed that *Kiss1* is a true ligand for GPR54. The activation of GPR54 results in a release of GnRH hormones from the hypothalamus. The GnRH hormones stimulate the release of LH and FSH from the anterior pituitary into the bloodstream, stimulating development of testis and ovaries. The image of the brain was obtained from http://web.bvu.edu/faculty/ferguson/Course_Material/BioPsych/Images1/Hypothalamus.jpg.

With this series of experiments, Tassigny et al., were able to fill the last piece of the GPR54/*Kiss1* puzzle. They showed that the *Kiss1* gene encodes a true ligand for the GPR54 receptor and that *Kiss1* does not possess any additional physiological roles (Figure 1). By comparing the phenotypes of *Kiss1*-/-mice and GPR54-/- mice, they were able to eliminate any other possible ligands for the GPR54 receptor since the phenotypes seen in both studies are the same. The only issue with this study is that the researchers did not repeat the GPR54 knockout mice experiments from previous studies. Including both of the phenotypes would strengthen the discovery.

Establishing the GPR54/*Kiss1* relationship allowed scientists to further investigate the mechanism through which kisspeptin activates GPR54 to regulate reproductive organ development. Liu et al., showed that activation of GPR54 by kisspeptin results in PLC-IP3R-calcium cascade, which regulated potassium and NSC channels to initiate depolarization in GnRH neurons, thereby releasing the hormones (10). Liu et al., suggest that understanding this mechanism can potentially lead to new treatments for inherited/congenital reproductive disorders. As well, they suggest possible novel treatments for sex hormone-dependent cancers. Kisspeptins were originally identified as a tumor metastasis suppressor gene (6). Interestingly, reduced expression levels of *Kiss1* have been found in metastatic cancers such as brain cancer (11). Thus, knowing the mechanism of action of the tumor metastasis suppressor gene will allow new insights into understanding sex hormone-dependent cancers.

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