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Expression of cyclin D3 transcripts in the postmeiotic male germ cells of the mouse

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D-type G1 cyclins are known to be crucial for the progression of mitotic cell cycle in mammals. It is, however, largely unknown whether D-type cyclins are directly involved in the regulation of meiotic germ cell development. In the present study, we examined the expression patterns of D-type cyclins (cyclin D1 and D3) during male germ cell development by Northern blot and *in situ* hybridization analyses. In the adult testes, we detected a 4.2 kb of cyclin D1 mRNA and two different sizes (2.3 kb and 1.8 kb) of cyclin D3 mRNAs. In particular, expression of a short form of cyclin D3 (1.8 kb) was testis-specific. During the testicular development, expression of cyclin D3 was increased and a 1.8 kb transcript first appeared along with a presentation of postmeiotic germ cells in the testis. On the other hand, expression of cyclin D1 gradually decreased with the testicular development. *In situ* hybridization approach also revealed that expression of cyclin D3 was restricted to the postmeiotic germ cells. Furthermore, the 2.3 kb transcript was highly expressed in round spermatids and marginally expressed in elongated spermatids/residual bodies, while the 1.8 kb transcript was more abundantly expressed in elongated spermatids/residual bodies. Sucrose-gradient separation of polysomal RNA fractions demonstrated that some portions of 2.3 kb transcripts were translationally active, while the 1.8 kb transcript was likely to be inactive. Taken together, the present study indicates a functional importance of cyclin D3 expression in the differentiated postmeiotic male germ cells.

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Buserelin, a Gonadotropin-Releasing Hormone (GnRH) Agonist, Strongly Suppresses the Rat GnRH Promoter Activity in Hypothalamic GT1-1 Neuronal Cells: Implication of Autocrine Regulation of GnRH Transcription

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To examine whether an ultrashort feedback mechanism operates at the level of gonadotropin-releasing hormone (GnRH) gene transcription, we studied the effects of GnRH and its analogs on the GnRH promoter activity in hypothalamic GT1-1 neuronal cells. Chronic treatment of GT1-1 cells for 24 h with native GnRH or buserelin, a GnRH agonist, significantly decreased GnRH promoter activity, whereas treatment with antide, a GnRH antagonist, showed little effect. The inhibitory effect of buserelin on GnRH gene transcription was dose-related, and significant inhibition was observed in groups treated with buserelin at a concentration higher than 0.1 μM. Time course experiment showed that significant decrease in GnRH promoter-driven luciferase activity was observed within 12 h and sustained up to 48 h. Promoter analysis with a serial deletion mutants revealed that buserelin-induced repression required the -0.5 kb upstream promoter region. Pretreatment with GnRH antagonists (1 or 10 μM), however, failed to reverse buserelin-induced decrease in GnRH gene expression, suggesting that the inhibitory effect of buserelin might be independent of GnRH receptor or that antagonists (10 μM) might be insufficient to antagonize the effect of buserelin (1 μM). Treatment of GT1-1 cells with 10 μM buserelin for 24 h decreased basal GnRH secretion and completely blocked K^+-induced GnRH secretion. These data clearly demonstrate that GnRH agonist, buserelin can exert its autocrine regulation primarily at the level of GnRH gene transcription.