Nitric oxide by cultured rat Leydig and Sertoli cells
- Cell-to-cell interaction in the testis-
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Nitric oxide (NO) is a free radical generated from L-arginine by NO synthase (NOS), and plays various roles in many biological systems. In the present study, we examined whether inducible NOS (iNOS) exists in cultured rat Leydig cells and whether Leydig cells produce NO. We also analyzed cell-to-cell interaction of NO production between germ cells and Leydig or Sertoli cells, and the relationship between NO and steroidogenesis.

Immature rat Leydig and Sertoli cells were isolated from 20-day-old S-D rats. Adult rat Leydig cells and germ cells (pachytene spermatocytes;PS and round spermatids;Rsd) were isolated from 8-week-old S-D rats. Leydig and Sertoli cells were cultured with cytokines (IL-1, TNFα, or forskolin), hormones (hCG or FSH), or germ cell-conditioned media for various periods up to 24h. NO was measured as nitrite in the supernatant of culture media using the method of Griess. iNOS mRNA was analyzed by Northern blot hybridization using iNOS cDNA probe. We also measured testosterone (T) in the supernatant of Leydig cell culture media using EIA.

NO production of immature rat Leydig cells was stimulated by IL-1β in a dose-dependent manner and not affected by TNFα, forskolin, nor hCG. iNOS mRNA expression of immature Leydig cells was increased by IL-1β in a dose-dependent manner. It showed the peak at 6h and was maintained up to 24h. NO production of adult Leydig cells was increased by IL-1β in a dose-dependent manner. iNOS mRNA expression of adult Leydig cells was recognized at 6h and 12h after the addition of IL-1β. NO production and iNOS mRNA expression of immature Leydig cells were increased by Rsd-conditioned medium in a dose-dependent manner and were not influenced by PS-conditioned medium. iNOS mRNA expression of Sertoli cells was increased by Rsd-conditioned medium in a dose-dependent manner and was not affected by IL-1β, TNFα, forskolin, FSH, nor PS-conditioned medium. NO donor, sodium nitropurusside inhibited steroidogenesis of Leydig cells in a dose-dependent manner. LH and PS-conditioned medium which stimulate T production did not increase NO production of Leydig cells. IL-1β which stimulates NO production inhibited T production induced by LH.

In conclusion, we showed that 1)rat Leydig cells expressed iNOS mRNA and produced NO, which were induced by IL-1β, 2)NO production and iNOS mRNA expression of rat Leydig and Sertoli cells were regulated by some factor(s) released from Rsd, and 3)NO produced by Leydig cells may inhibit steroidogenesis of themselves.