

# Testicular endocrine activity is upregulated by D-aspartic acid in the green frog, *Rana esculenta*

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## Abstract

This study investigated the involvement of D-aspartic acid (D-Asp) in testicular steroidogenesis of the green frog *Rana esculenta* and its effect on stimulation of thumb pad morphology and glandular activity, a typical testosterone-dependent secondary sexual characteristic in this amphibian species. In the testis, D-Asp concentrations vary significantly during the reproductive cycle: they are low in pre- and post-reproductive periods, but reach peak levels in the reproductive period (140-236 nmol/g wet tissue). Moreover, the concentrations of D-Asp in the testis through the sexual cycle positively match the testosterone levels in the gonad and the plasma. The racemase activity evaluated during the cycle expresses its peak when D-Asp and testosterone levels are highest, that is, during the reproductive period, confirming the synthesis of D-Asp from L-Asp by an aspartate racemase. Short-term *in vivo* experiments consisting of a single injection of D-Asp (2.0 micro mol/g body weight) demonstrated that this amino acid accumulates significantly in the testis, and after 3 h its uptake is coupled with a testosterone increase in both testis and plasma. Moreover, within 18 h of amino acid administration, the D-Asp concentration in the testis decreased along with the testosterone titer to prestimulation levels. Other amino acids (L-Asp, D-Glu and L-Glu) used instead of D-Asp were ineffective, confirming that the significant increase in testicular testosterone was a specific feature of this amino acid. In long-term experiments, D-Asp had been administered chronically to frogs caught during the three phases of the reproductive cycle, inducing testosterone increase and 17beta-estradiol decrease in the gonad during the pre- and post-reproductive period, and vice versa during the reproductive period. The stimulatory effect of D-Asp on testosterone production by the testis is consistent with the stimulation of spermatogenesis and the maturation of thumb pads occurring in D-Asp-treated frogs. In these last animals, there was an increase of seminiferous ampoule area and a higher number of spermatids and sperm. Moreover, in spermatogonia I and II and in spermatocytes, a proliferating cell nuclear antigen (PCNA) intense immunopositivity was observed. In addition, the thumb pads of D-Asp-treated frogs compared with controls showed a significantly thicker epithelial lining, a wider area of their glands with taller secretion cells, and more numerous, PAS-positive-rich secretions. Finally, these results provide functional evidence for a biologic role of D-Asp in amphibian male steroidogenesis; therefore, this unusual amino acid could be considered a modulatory agent for reproductive processes.