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Stage-Specific GDNF Expression by Rat Sertoli Cells: A Possible Regulator of the Replication and Differentiation of Stem Spermatogonia

Supplemental Method

Methods used in initial attempts to examine distribution of GDNF in cross sections of paraffin-embedded rat testes.

Testes of 90-120 day old rats were fixed in Bouins fixative or in 4% paraformaldehyde via whole body perfusion. Testes were cut into three parts and embedded in paraffin using standard procedures. Five micron sections were cut from the embedded tissue and the sections were dewaxed and rehydrated. Saturated Lithium carbonate was used to remove picric acid from the tissue sections fixed in Bouins fixative. Then, testes sections were incubated for one hour in blocking solution (2% BSA, 0.4% Triton X-100 in Tris-saline) and the testes sections were incubated overnight at 4° C in primary antibody or nonimmune IgG. We used either goat anti-GDNF (R&D Systems, Minnneapolis, MN), goat IgG (Sigma-Aldrich, St. Louis MO, , rabbit anti-GDNF (Santa Cruz Biotechnology, Santa Cruz, CA) or Protein A-purified rabbit IgG. Antibodies were diluted in blocking solution at dilutions from 1:100 to 1:400. Control IgGs were used at an equivalent concentration. After washing of the sections in blocking solution, the tissue sections were then incubated overnight at 4ºC either in 1:400 Alexa Flor 488 donkey anti goat IgG or Alexa fluor 488 goat anti-rabbit IgG (Invitrogen, San Diego CA). Following further washes in blocking solution, the tissue sections were mounted in Vectashield (Vector Laboratories, Burlingame, CA) and examined by standard fluorescence microscopy.