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Nerve growth factor mRNA and protein in the testis and epididymis of mouse and rat

(germ cells/*in situ* and blot hybridization/nerve growth factor receptor mRNA/immunohistochemistry/bioassay)

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ABSTRACT ***In situ*** **hybridization using β-nerve growth factor (NGF) DNA probes was used to demonstrate NGF mRNA in spermatocytes and early spermatids of adult mouse. NGF mRNA-containing cells were also identified in the epithelium of convoluted ducts in mouse corpus epididymidis. Blot-hybridization analysis of RNA prepared from mouse testis and epididymis as well as from rat epididymis confirmed the presence of a 1.3-kilobase (kb) NGF mRNA in these tissues. In the rat testis, however, only a 1.5-kb NGF mRNA was found, corresponding in size to a minor NGF mRNA detected in the rat brain, heart, and epididymis. By using affinity-purified anti-NGF antibodies, NGF-like immunoreactivity was observed in germ cells of rat and mouse testis and in the lumen of epididymis. Extracts of both mouse epididymis and testis stimulated fiber outgrowth in cultured sympathetic ganglia, and the effect was blocked by antibodies to mouse NGF. A two-site enzyme immunoassay showed the presence of 10 and 70 ng of NGF per g of tissue in the mouse testis and epididymis, respectively. Furthermore, RNA blot analysis showed the presence of mRNA for the NGF receptor in mouse testis. These results suggest a nonneurotrophic role for NGF in the male reproductive system, possibly in survival maturation and/or motility of spermatozoa.**

β-Nerve growth factor (NGF) is a protein essential for the development and maintenance of sensory and sympathetic neurons in the peripheral nervous system (1, 2). Recently RNA blot analysis, enzyme-linked immunoassays, and immunohistochemistry have been used to demonstrate NGF mRNA and protein in the mammalian brain (3–6), where NGF has been suggested to support basal forebrain cholinergic neurons (7–10). The levels of NGF mRNA in most peripheral tissues as well as in the brain appear to correlate with their degree of innervation by NGF-sensitive fibers either sympathetic in the periphery or cholinergic in the central nervous system (3–6, 11, 12).

In the male mouse submandibular gland, which constitutes an extremely rich source of NGF mRNA and protein, the level of NGF synthesis does not correlate, however, with the innervation by NGF-sensitive fibers (1). Also, relatively high levels of NGF protein have been found in the snake venom gland (1), in guinea pig and rabbit prostate (13–16), and in bull semen and seminal vesicle (17, 18). Furthermore, in the mouse testis, NGF-like immunoreactivity was recently shown to be present in germ cells, and a possible role for NGF in sperm maturation or motility was hypothesized (19).

In the study reported here, we used a mouse NGF cDNA probe for *in situ* hybridization and RNA blot analysis to reveal the identity and distribution of cells synthesizing NGF in rodent male genital organs. The presence of NGF protein in the same tissues was studied by immunohistochemistry, immunoassays, and biological tests of activity. Our results show that male mouse germ cells synthesize both NGF mRNA and protein. In addition, NGF mRNA-containing cells were found in the epithelium of convoluted ducts of corpus epididymidis. These results together with the demonstration of NGF receptor mRNA in mouse testis suggest a role for NGF in maturation or motility of spermatozoa or both.

EXPERIMENTAL PROCEDURES

In Situ **Hybridization.** Adult mouse and rat males were anesthetized with pentobarbital (Mebumal vet. ACO Sweden; 0.06 mg/g of body weight) and perfused through the aorta with 4% paraformaldehyde in 0.1 M Sörensen's buffer (pH 7.4). Pieces of tissue, including testis and epididymis, were dissected and postfixed by immersion in the same fixative for 2 hr and then rinsed in 5% sucrose in the same buffer. Sections were cut at 8 μm on a cryostat, thawed on gelatin-coated slides, and kept frozen at −80°C. The conditions for *in situ* hybridization were essentially as described by Bloch *et al.* (20). A 900-base-pair (bp) *Pst* I fragment derived from a mouse NGF cDNA clone (21, 22) was used as a probe for NGF mRNA. Plasmid pUC9 was used as a control probe. The DNA probes were labeled with [^{3}H]dCTP by nick-translation to a specific activity of around 10^7 cpm/μg. After hybridization, the slides were rinsed briefly in 4× SSC (1× SSC is 0.15 M NaCl/0.015 M sodium citrate, pH 7.0) and washed once in 2× SSC at room temperature, once in 0.5× SSC at 35°C, and finally in 0.1× SSC at 35°C. The slides were dipped in 50% Kodak NTB2 nuclear emulsion and exposed for 3 months in a dry chamber at 4°C. After development the sections were lightly counterstained with Erlich's hematoxylin and mounted. In a few cases, the probes were labeled with [^{32}P]dCTP to a specific activity of approximately 10^9 cpm/μg. The Ilford K5 emulsion was then used for dipping of the slides, which were exposed for 5–10 days. The results of the hybridizations were quantified by using a computerized image-analysis system (IBAS, Zeiss/Kontron). The labeling of cells was evaluated as the percentage of the area over defined cells that was covered with silver grains. Background labeling was measured as the percentage of labeled surface in defined areas of the same section outside the cells of interest. In each region of a section, 18–24 measurements were made and repeated in four different slides.

RNA Blot-Hybridization Analysis. Polyadenylylated RNA was prepared from the indicated tissues by disruption in 4 M guanidine isothiocyanate, followed by centrifugation through

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Abbreviation: NGF, nerve growth factor.

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CsCl (23) and oligo(dT)-cellulose chromatography (24). Polyadenylylated RNA (10 μg) from each sample was electrophoresed in a 1% agarose gel containing 0.7% formaldehyde. The gel was blotted onto nitrocellulose and hybridized to a 900-bp *Pst* I fragment derived from a mouse NGF cDNA clone (21, 22) or to a 400-bp *Bst*E2 fragment derived from a rat NGF receptor cDNA clone (25). Both fragments were labeled with [^{32}P]dCTP by nick translation to a specific activity of approximately 10^9 cpm/μg. The filters were washed at high stringency (0.1× SSC/0.1% $NaDodSO_4$ at 54°C) and exposed to x-ray films at −80°C with a DuPont intensifying screen.

Immunohistochemistry. Cryostat sections, 14 μm thick, for indirect immunocytochemistry (26) were made from tissues of animals fixed by perfusion with 4% paraformaldehyde/0.3% picric acid. Sections were first incubated with affinity-purified sheep anti-mouse NGF antibodies (20 μg/ml of phosphate-buffered saline) (27) and then by fluorescein isothiocyanate-labeled antibodies to sheep immunoglobulin. For control, anti-mouse NGF antibodies were preadsorbed on purified mouse NGF (5 μg/ml) for 24 hr at 4°C.

Assays for NGF Protein and Activity. The biological assay for NGF activity was performed by using chicken embryo sympathetic ganglia as described by Ebendal *et al.* (28). NGF-like immunoreactivity was determined by a fluorometric two-site enzyme immunoassay (29).

RESULTS

Identification of NGF mRNA-Containing Cells in Testis and Epididymis. Cells producing NGF mRNA in the adult mouse testis and epididymis were identified by *in situ* hybridization. The distribution of radioactive material was similar using ^{32}P- or ^{3}H-labeled probes. However, only the ^{3}H-labeled probes gave a resolution at the level of individual cells. In the mouse testis, labeling was obtained in the seminiferous tubules with a concentration of silver grains overlaying the cytoplasm of germ cells (Fig. 1*a*). The labeled cells had the typical shape, size, and localization of spermatocytes and early spermatids. Interstitial Leydig cells showed no labeling, and Sertoli cells also seemed negative. Similarly, no labeling was obtained over spermatogonia localized in the basal part of the tube or over maturating spermatids and spermatozoa in the central part of the seminiferous tubules. No cell type in the mouse testis was labeled when a control plasmid was used as a probe (Fig. 1*b*) or after pretreatment of the sections with RNase A. The density of labeling of spermatocytes was determined by using a computerized image analysis and ranged from 22 to 86 times the background level. The density of labeling in positive cells was approximately 30 times higher than after hybridization with the control plasmid probe.

The NGF probe showed strong labeling of sections from corpus epididymidis, with silver grains concentrated over pseudostratified columnar epithelial cells in the duct (Fig. 1*c*). Labeling was restricted to the principal cells of the epithelium, with no labeling above the background in basal cells. Labeled cells were sparse in the epithelium of cauda epididymidis. As for the testis, no labeling was seen when a plasmid control probe was used (data not shown) or after pretreatment of the sections with RNase A (Fig. 1*d*).

Detection of NGF mRNA by RNA Blot Analysis. The presence of NGF mRNA in both testis and epididymis of mouse and rat was confirmed by RNA blot analysis. The epididymis of both species contained a similar level of a 1.3-kilobase (kb) NGF mRNA (Fig. 2). The levels of NGF mRNA in both mouse and rat epididymis were comparable to the level found in rat heart. The mouse testis contained NGF mRNA species of 1.3 kb and 1.5 kb, respectively, as well as two larger hybridizing RNA species. In the rat testis, only a minor 1.5-kb NGF mRNA was detected.

Localization of NGF-Like Immunoreactivity. With affinity-purified antibodies raised against NGF, a positive reaction was observed in germ cells of all stages from primary spermatocytes to mature spermatozoa in the seminiferous tubules of both rat and mouse testis. An intensive fluorescence was observed in the cytoplasm of primary and secondary spermatocytes, whereas no fluorescence was seen in spermatogonia (Fig. 3 *a* and *b*). Leydig cells in interstitial tissue were also nonimmunoreactive. Different seminiferous tubules containing germ cells at different stages of their

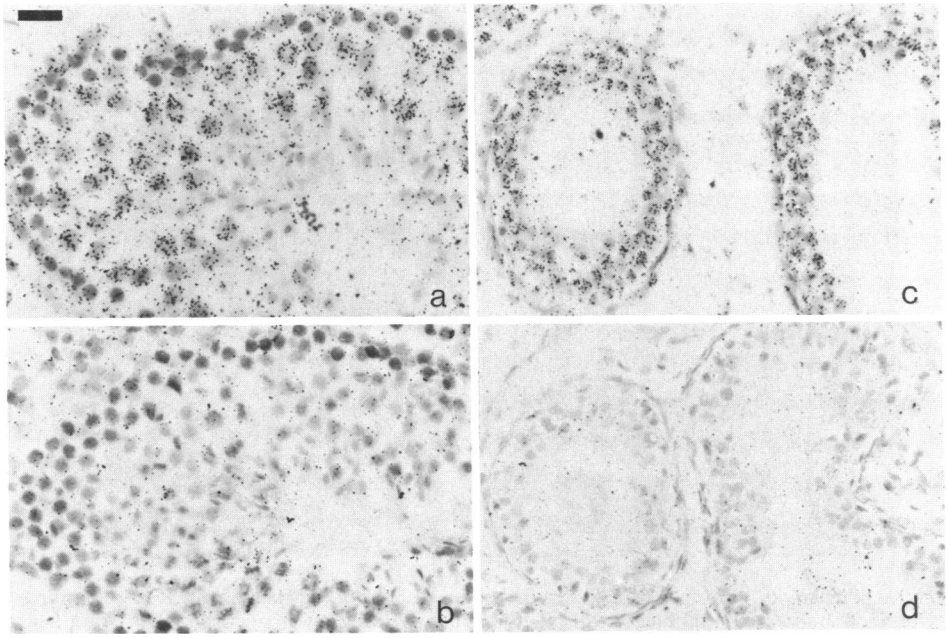

FIG. 1. *In situ* hybridization to sections from mouse testis and corpus epididymidis. Sections of mouse testis (*a*) or corpus epididymidis (*c* and *d*) were hybridized to a NGF cDNA probe labeled with [^{3}H]dCTP by nick-translation. (*a*) Labeled spermatocytes and early spermatids in seminiferous tubules. (*b*) Section adjacent to the one shown in *a*, hybridized with a plasmid control probe. (*c*) Epithelial cells in corpus epididymidis labeled after hybridization to the NGF probe. (*d*) Similar section as in *c*, pretreated with RNase A. (Bar = 20 μm.)

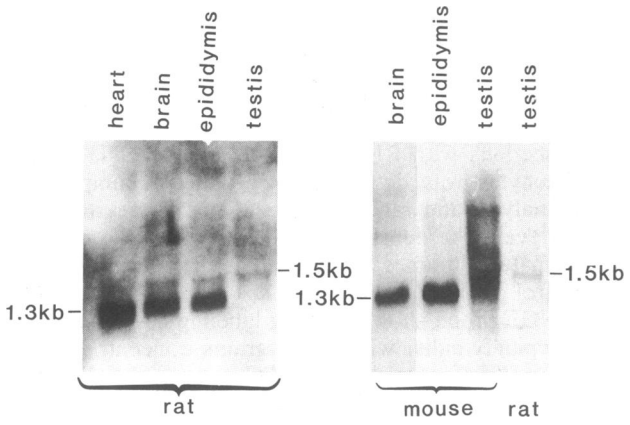

FIG. 2. RNA blot-hybridization analysis of NGF mRNA in mouse and rat tissues. Polyadenylylated RNA (10 μg) from the indicated tissues were separated on a formaldehyde-containing agarose gel, blotted onto nitrocellulose, and hybridized with a NGF DNA probe. The filter was washed at high stringency, followed by autoradiography.

development showed slightly different distribution of NGF-like immunoreactive material. Some tubules contained NGF-like immunoreactivity almost exclusively in primary and secondary spermatocytes, whereas others showed immunoreactive material also in the central parts of the ducts, corresponding to cells at later stages of spermatogenesis. In epididymis of both species, immunoreactive material was found in the duct lumen of caput, corpus (Fig. 3*c*), and cauda as well as in the ductus deferens. The immunoreactivity was observed mainly in sperm cells and in the form of small bodies resembling the immunoreactive residual bodies also seen in testis. The immunoreactive signal in epithelial cells of the various compartments of epididymis was weak.

Preadsorption of the NGF antibody with NGF purified from the male mouse submandibular gland abolished the fluorescence in testis and epididymis.

Presence of Biologically Active NGF. Testis and epididymis from adult mice were homogenized in tissue culture medium, and the extracts were added to explants of sympathetic ganglia dissected from 9-day chicken embryos. Cultures were examined with dark-field microscopy and scored for fiber outgrowth after 2 days of incubation. Both mouse extracts caused a significant stimulation of fiber outgrowth from the sympathetic ganglia (Fig. 4*a*; data not shown for the testis). The fiber outgrowth was completely blocked by two different antibodies raised against mouse NGF (Fig. 4*b*). The stimulation of fiber outgrowth indicated the presence of 13 ng of NGF per g of testis and 120 ng of NGF per g of epididymis when compared to the activity of known amounts of NGF purified from male mouse submandibular gland. A two-site enzyme immunoassay likewise showed the presence of 10 and 70 ng of NGF per g of testis and epididymis, respectively.

Detection of NGF Receptor mRNA. A DNA fragment from the recently isolated cDNA clone for the rat NGF receptor (25) was used as a hybridization probe to investigate the possible synthesis of NGF receptor mRNA in both testis and epididymis. This analysis showed the presence of a 3.4-kb NGF receptor mRNA in mouse testis (Fig. 5). The size of this NGF receptor mRNA was slightly smaller than the NGF receptor mRNA detected in the mouse brain (3.7 kb). The mouse epididymis contained a low level of a 3.7-kb NGF

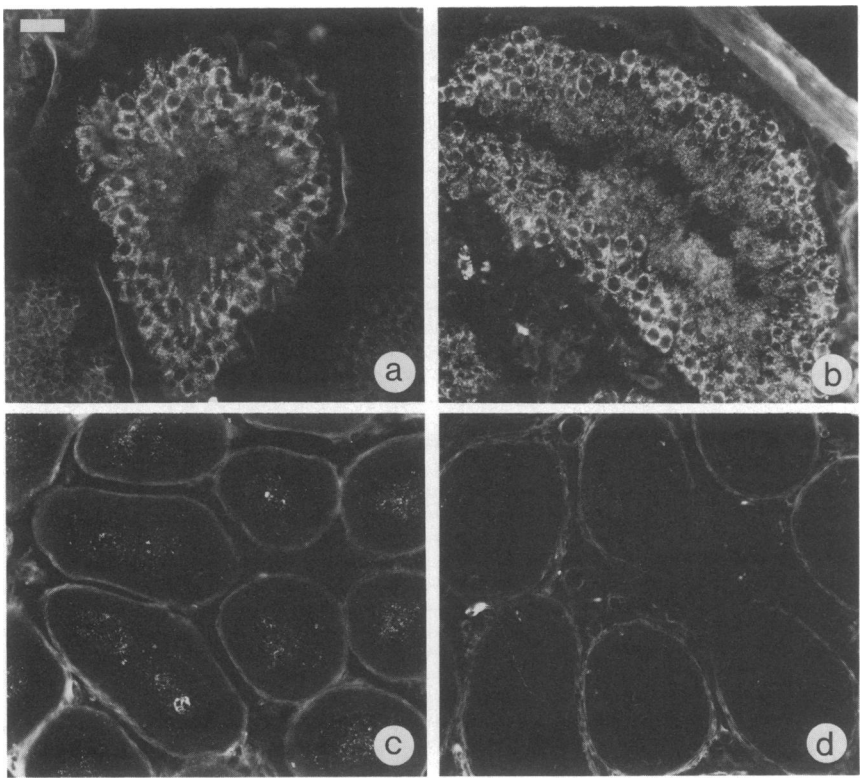

FIG. 3. NGF-like immunoreactivity in testis and epididymis. Sections of rat (*a*) and mouse (*b*) testis and mouse epididymis (*c*) were incubated with affinity-purified mouse NGF antibodies. (*a*) NGF-like immunoreactivity in spermatocytes of rat seminiferous tubules. The spermatogonia located between the outer spermatocytes and the autofluorescent basal membrane are negative. (*b*) NGF-like immunoreactivity in mouse seminiferous tubules. In addition to the reactivity seen in the spermatocytes, this tubule also shows immunoreactivity towards the lumen of the duct. Note that the interstitial Leydig cells (lower left) are nonfluorescent. (*c*) Immunoreactivity in corpus epididymidis of mouse. In the duct lumen, specific fluorescence is confined to sperm cells and possibly remnants of residual bodies. Fluorescence in the duct wall is unspecific, as illustrated in an adjacent section (*d*) treated only with the second antiserum. (Bar = 25 μm.)

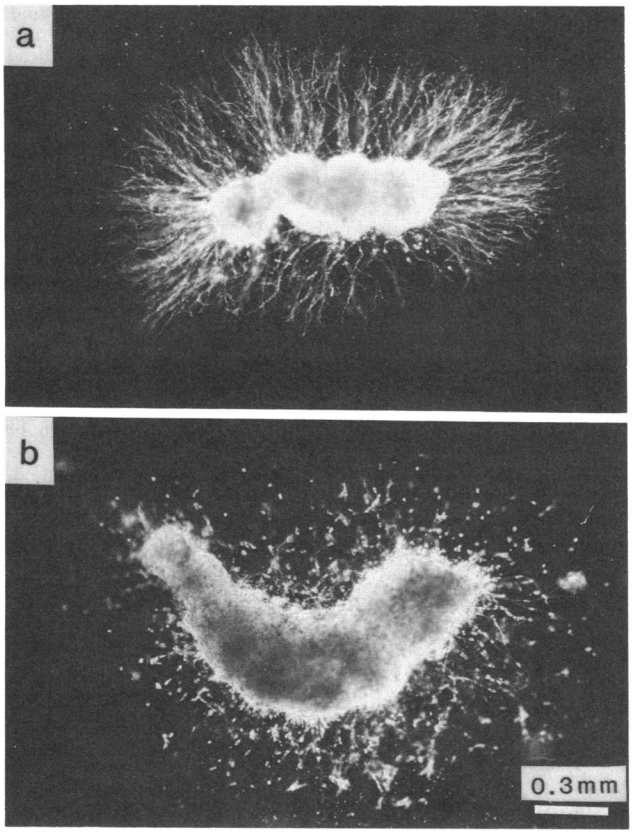

FIG. 4. Biological assay for NGF activity. Sympathetic ganglia from a 9-day chicken embryo were incubated *in vitro* in the presence of an extract from mouse epididymis. These dark-field micrographs show stimulation of fiber outgrowth by the extract (*a*) and block of the fiber response by addition of affinity-purified anti-NGF antibodies at 2 μg/ml (*b*). (Bar = 0.3 mm.)

receptor mRNA, whereas the level of NGF receptor mRNA in the mouse testis was clearly higher than in brain.

DISCUSSION

In this report we document the presence of NGF mRNA and protein in the rodent male reproductive system by using *in situ* hybridization, RNA blot-hybridization analysis, immunohistochemistry, enzyme-linked immunoassay, and a test of biological activity.

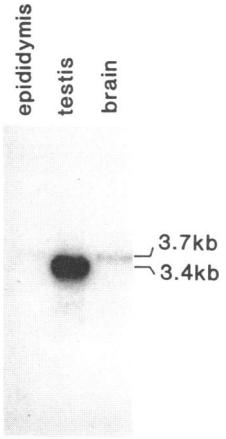

FIG. 5. RNA blot-hybridization analysis of NGF receptor mRNA. Polyadenylylated RNA (10 μg) from the indicated mouse tissues was separated on an agarose gel, blotted onto nitrocellulose, and hybridized with a 400-bp *Bst*E2 fragment from a rat NGF receptor cDNA clone. The filter was washed at high stringency, followed by autoradiography.

NGF mRNA-producing cells were identified in the mouse testis and epididymis by *in situ* hybridization. RNA blot analysis confirmed the presence of NGF mRNA in these tissues. Immunohistochemistry revealed the presence of NGF-like immunoreactivity in the same cell types as identified by *in situ* hybridization to synthesize NGF mRNA in the testis. This correlation strongly suggests that our *in situ* hybridization procedure with a NGF cDNA probe showed the correct hybridization specificity for NGF mRNA. A correct specificity of the *in situ* hybridizations was further suggested by the absence of labeling with either a control plasmid probe or after pretreatment of the sections with RNase A.

The NGF mRNA detected in rat testis was approximately 200 nucleotides longer than in the male mouse submandibular gland and corresponded in size to a minor NGF mRNA detected in several adult rat tissues, including heart and brain (4, 5, 11). The longer NGF mRNA can be the result of an alternative transcription start, an alternative splicing of a primary NGF transcript, or a difference in the length or position of the poly(A) tail. A single consensus hexanucleotide (ATTAAA) specifying polyadenylylation is found at the 3′ end of the rat NGF gene (30). Therefore, alternative use of polyadenylylation signals is unlikely to account for the longer NGF mRNA species in the testis. Alternative splicing of NGF transcripts, which in addition to the 1.3-kb mRNA also generates a shorter NGF mRNA, has been described (31). A similar mechanism of alternative RNA processing may explain the longer NGF mRNA seen in testis. Since in the rat testis only the 1.5-kb NGF mRNA species was detected, it should be an appropriate tissue to study the processing and structure of this alternative NGF mRNA.

Biologically active NGF, as assayed with chicken embryo ganglia *in vitro*, has been detected in semen and prostate gland from several mammals, including guinea pig (13), rabbit (15), shrew (32), and bull (15, 17). However, the cells responsible for NGF synthesis in the male reproductive system of these species remain to be identified. Parts of the male reproductive system, especially vas deferens, are densely innervated by sympathetic fibers. As shown for NGF expression in other peripheral tissues, the high density of sympathetic innervation in vas deferens results in a high level of NGF mRNA (11, 12). With *in situ* hybridization, only endothelial cells of blood vessels have been reported to be labeled in vas deferens (33). In contrast to NGF expression in vas deferens, the high levels of NGF mRNA in testis and epididymis of rat and mouse do not correlate with the density of sympathetic innervation. In these tissues NGF mRNA was localized to germ cells and epididymis epithelial cells.

NGF mRNA and NGF-like immunoreactivity was detected in spermatocytes and early spermatids. The NGF-like immunoreactivity previously demonstrated in mouse testis (19) has now been shown to be present also in the rat testis. In addition, immunoreactivity also was found in the lumen of ductus epididymidis, where epithelial secretion products are released. Only weak immunoreactivity was found in the epithelial cells of epididymis. A stronger immunoreactivity might be expected with antibodies directed against peptides derived from the pro-NGF protein.

The highest levels of NGF mRNA were found in the epididymis, mainly in lower caput and in corpus. The synthesis of NGF mRNA in epithelial cells of the convoluted tubule of corpus epididymidis further suggests that sperm cells require NGF for proper maturation and migration. The spermatozoa show anarchic flagellar motility in the proximal part of caput epididymidis, and they only acquire forward motility while migrating through epididymis (34–39). The

change in motility pattern depends on factors produced by the epididymis epithelium and present in the plasma of epididymis (40). These factors can be adsorbed on spermatozoa during their migration through epididymis (40). A specific adsorption of NGF on migrating spermatozoa requires the presence of the NGF receptor on these cells. Indeed, a high level of NGF receptor mRNA was detected in mouse testis. Thus, it is possible that the NGF receptor is expressed on germ cells, although the cells responsible for NGF receptor synthesis remain to be identified. If the presence of the NGF receptor on mature spermatozoa is confirmed, an autocrine regulation of spermatogenesis by NGF is likely in similarity with the autocrine control of cell growth described for platelet-derived growth factor (41, 42). In addition, NGF immunoreactivity was also found in other areas of the reproductive system. No NGF mRNA was found in the lumen of seminiferous tubules, but in some tubules NGF-like immunoreactivity was seen in mature spermatozoa. This suggests that NGF mRNA is translated at an early stage of germ-cell maturation, producing a NGF protein that remains at all later stages of spermatogenesis. Therefore, a function for the NGF protein at a late stage of spermatogenesis may be postulated. NGF-like immunoreactivity was also present on spermatozoa in the lumen of cauda epididymidis and ductus deferens. The detection of NGF in sperm cells at various stages of their maturation may suggest that sperm cells, like neurons (1), need NGF for survival. The presence of NGF mRNA and protein in male germ cells and NGF mRNA in epithelial cells supporting germ cell maturation and migration strongly suggest a nonneurotrophic role for NGF during sperm maturation. However, further experiments are necessary to reveal the mechanisms by which NGF could influence sperm cells.

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1. Levi-Montalcini, R. & Angeletti, P. U. (1986) *Physiol. Rev.* **48,** 534–565.
2. Thoenen, H. & Barde, Y. A. (1980) *Physiol. Rev.* **60,** 1284–1335.
3. Korsching, S., Auburger, G., Heumann, R., Scott, J. & Thoenen, H. (1985) *EMBO J.* **4,** 1389–1393.
4. Whittemore, S. R., Ebendal, T., Lärkfors, L., Olson, L., Seiger, Å., Strömberg, I. & Persson, H. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 817–821.
5. Shelton, D. L. & Reichardt, L. F. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 2714–2718.
6. Goedert, M., Fine, A., Hunt, S. P. & Ullrich, A. (1986) *Mol. Brain Res.* **1,** 85–92.
7. Gnahn, H., Hefti, F., Heumann, R., Schwab, M. E. & Thoenen, H. (1983) *Dev. Brain Res.* **9,** 45–52.
8. Mobley, W. C., Rutkowski, J. L., Tehnnekoon, G. I., Buchanan, K. & Johnston, M. U. (1985) *Science* **229,** 284–287.
9. Large, T. H., Bodary, S. C., Clegg, D. O., Weskamp, G., Otten, U. & Reichardt, L. F. (1986) *Science* **234,** 352–355.
10. Hefti, F., Hartikka, J., Eckenstein, F., Gnahn, H., Heumann, R. & Schwab, M. E. (1985) *Neuroscience* **14,** 55–68.
11. Shelton, D. L. & Reichardt, L. F. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 7951–7955.
12. Heumann, R., Korsching, S., Scott, J. & Thoenen, H. (1984) *EMBO J.* **3,** 3183–3189.
13. Harper, G. P., Barde, Y. A., Burnstock, G., Carstairs, J. R., Dennison, M. E., Suda, K. & Vernon, C. A. (1979) *Nature (London)* **279,** 160–162.
14. Chapman, C. A., Banks, B. E. C., Carstairs, J. R., Pearce, F. L. & Vernon, C. A. (1979) *FEBS Lett.* **105,** 341–344.
15. Harper, G. P. & Thoenen, H. (1980) *J. Neurochem.* **34,** 893–903.
16. Rubin, J. S. & Bradshaw, R. A. (1981) *J. Neurosci. Res.* **6,** 451–464.
17. Harper, G. P., Glanville, R. W. & Thoenen, H. (1982) *J. Biol. Chem.* **257,** 8541–8548.
18. Hofmann, H. D. & Unsicker, K. (1982) *Eur. J. Biochem.* **128,** 421–426.
19. Olson, L., Ayer-LeLievre, C., Ebendal, T. & Seiger, Å. (1987) *Cell Tissue Res.* **248,** 275–286.
20. Bloch, B., Milner, B. J., Baird, A., Gubler, U., Reymond, C., Boblen, P., LeGuellec, D. & Bloom, F. E. (1984) *Regulat. Peptides* **8,** 345–354.
21. Scott, J., Selby, M., Urdea, M., Quiroga, M., Bell, B. & Rutter, W. J. (1983) *Nature (London)* **302,** 538–540.
22. Ullrich, A., Gray, A., Berman, C. & Dulh, T. J. (1983) *Nature (London)* **303,** 821–825.
23. Chirgwin, J., Przybyla, A., MacDonald, R. & Rutter, W. (1979) *Biochemistry* **18,** 5294–5299.
24. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1408–1412.
25. Radeke, M. J., Misko, T. P., Hsu, C., Herzenberg, L. A. & Shooter, E. M. (1987) *Nature (London)* **325,** 593–597.
26. Coons, A. H. (1958) in *General Cytochemical Methods*, ed. Danielli, J. F. (Academic, New York), pp. 399–422.
27. Ebendal, T., Olson, L. & Seiger, Å. (1983) *Exp. Cell Res.* **148,** 311–317.
28. Ebendal, T., Olson, Seiger, Å. & Belew, M. (1984) in *Cellular and Molecular Biology of Neural Development*, ed. Black, I. B. (Plenum, New York), pp. 231–242.
29. Lärkfors, L. & Ebendal, T. (1987) *J. Immunol. Methods* **97,** 41–47.
30. Whittemore, S. R., Persson, H., Ebendal, T., Lärkfors, L., Larhammar, D. & Ericsson, A. (1987) *NATO Advanced Study Institutes Series: Life Sciences* (Springer, Berlin), in press.
31. Edwards, R. H., Selby, M. J. & Rutter, W. J. (1986) *Nature (London)* **319,** 784–786.
32. Ueyama, T., Saito, H. & Yohro, T. (1981) *Biomed. Res.* **2,** 438–441.
33. Goedert, M. & Hunt, S. P. (1986) in *In Situ Hybridization in Brain*, ed. Uhl, G. R. (Plenum, New York), pp. 151–169.
34. Brandt, H., Acott, T. S., Johnson, D. J. & Hoskins, D. D. (1978) *Biol. Reprod.* **19,** 830–835.
35. Hoskins, D. D., Brandt, H. & Acott, T. S. (1978) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37,** 2534–2542.
36. Hoskins, D. D., Acott, T. S., Critchlow, L. & Vipayaraghavan, S. (1983) *J. Submicrosc. Cytol.* **15,** 21–27.
37. Acott, T. S. & Hoskins, D. D. (1981) *Biol. Reprod.* **24,** 234–240.
38. Kann, M. L. & Serres, C. (1980) *Reprod. Nutr. Dev.* **20,** 1739–1749.
39. Serres, C. & Kann, M. L. (1984) *Reprod. Nutr. Dev.* **28,** 81–94.
40. Cooper, T. G. (1986) in *The Epididymis Sperm Maturation and Fertilisation* (Springer, Berlin), Vol. 3, pp. 202–230.
41. Goustin, A. S., Betsholtz, C., Pfeifer-Ohlsson, S., Persson, H., Rydnert, J., Bywater, M., Holmgren, G., Heldin, C.-H., Westermark, B. & Ohlsson, R. (1985) *Cell* **41,** 301–312.
42. Betsholtz, C., Westermark, B., Ek, B. & Heldin, C.-H. (1984) *Cell* **39,** 447–457.