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Estrogen Differentially Regulates Estrogen and Nerve Growth Factor Receptor mRNAs in Adult Sensory Neurons

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We have previously shown that neurons in the basal forebrain colocalize the neurotrophin receptor p75^NGFR^ and estrogen receptors. The present study was designed to examine (1) if neural neurotrophin targets respond to estrogen as a general phenotypic feature and (2) if NGF receptor mRNAs are regulated by estrogen, using a prototypical target of NGF, the dorsal root ganglion (DRG) (sensory) neuron. We demonstrate, for the first time, the presence of estrogen receptor mRNA and protein (binding sites) in adult female rat DRG. Moreover, estrogen receptor mRNA expression, while present in DRG neurons from both the ovariectomized (OVX; estrogen deficient) and intact female rat, was *down-regulated,* as in the adult CNS, during proestrus (high estrogen levels) and in OVX animals replaced with proestrus levels of estrogen, as compared to OVX controls. In contrast, although the mRNAs for the NGF receptors p75^{NGFR} and *trkA* were also expressed in DRG neurons from OVX and intact animals, expression of both NGF receptor mRNAs was *up-regulated* in sensory neurons during proestrus, as compared to the OVX condition. Estrogen replacement, on the other hand, resulted in a transient *downregulation* of p75^{NGFR} mRNA and a time-dependent *upregulation* of *trkA* mRNA. Estrogen regulation of NGF receptor mRNA in adult peripheral neural targets of the neurotrophins supports the hypothesis that estrogen may regulate neuronal sensitivity to neurotrophins such as NGF and may be an important mediator of neurotrophin actions in normal neural function and following neural trauma.

[Key words: estrogen receptors,* trkA *mRNA, p75^{NGFR} mRNA, dorsal root ganglia, proestrus, ovariectomy, estrogen replacement]

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Exposure to estrogen profoundly influences developing neural cytoarchitecture and organization, as well as the expression and maintenance of neuroendocrine and behavioral functions and systems in adulthood. Growth- (neurite-) promoting effects of estrogen were first demonstrated in organotypic explant cultures of the developing rodent hypothalamus, preoptic area, and cerebral cortex (Toran-Allerand, 1976, 1980, 1984; Toran-Allerand et al., 1980, 1983), and subsequently confirmed in the developing brain *in situ* (Nishizuka and Arai, 1981; Hammer and Jacobson, 1984; Stanley and Fink, 1986; Stanley et al., 1986; Lustig et al., 1991) or as transplants in the eye (Nishizuka and Arai, 1982) or brain (Matsumoto et al., 1988) of adult hosts, as well as in estrogen target regions of the deafferented (Matsumoto and Arai, 1981) or steroid-deprived (Frankfurt et al., 1990) adult CNS.

Estrogen regulates the transcription of a variety of cytoskeletal proteins (Guo and Gorski, 1988; Lustig et al., 1991), steroid, peptide, and neurotransmitter receptors (Dohanich et al., 1982; MacLusky and McEwen, 1978; DeKloet et al., 1986), as well as hormones and neuropeptides (Wilcox and Roberts, 1985; Romano et al., 1988), although the mechanism of estrogen action on many of its target genes is poorly understood. The estrogen receptor is a member of the superfamily of steroid/thyroid hormone/vitamin D_3/retinoic acid receptors capable of activating genes by directly binding DNA sites containing hormone-specific regulatory elements (Evans, 1988; Beato, 1989; O'Malley, 1990). An estrogen response element (ERE) has been identified in several estrogen-responsive genes including vitellogenin (Klein-Hitpass et al., 1986), c-*fos* (Weisz and Rosales, 1990), prolactin (Watermann et al., 1988), and β-luteinizing hormone (Shupnik and Rosenzweig, 1991), suggesting that, in some instances, steroid effects may be mediated through direct activation of relevant genes. Alternatively, estrogen-inducible genes may be regulated through intermediate steps via interactions with, or secondary activation of, endogenous transcription-regulating growth factors or their receptors, such as the neurotrophin family of NGF-related peptides and their receptors. Analysis of the identified promoter region (Sehgal et al., 1988) of the p75^{NGFR} (Toran-Allerand et al., 1992a) and 5′-flanking region of the *trkA* genes indicates the presence of sequences with a high degree of homology to the putative vitellogenin and c-*fos* EREs (Fig. 1), consistent with the hypothesis that, in some instances, estrogen may regulate steroid-inducible genes through interactions with the neurotrophins or their receptors.

Two NGF receptors have been described. The 75 kDa protein p75^{NGFR} (Chao et al., 1986; Johnson et al., 1986; Radeke et al., 1987) has been shown to bind with low affinity at least three members of the neurotrophin family; NGF, brain-derived neu-

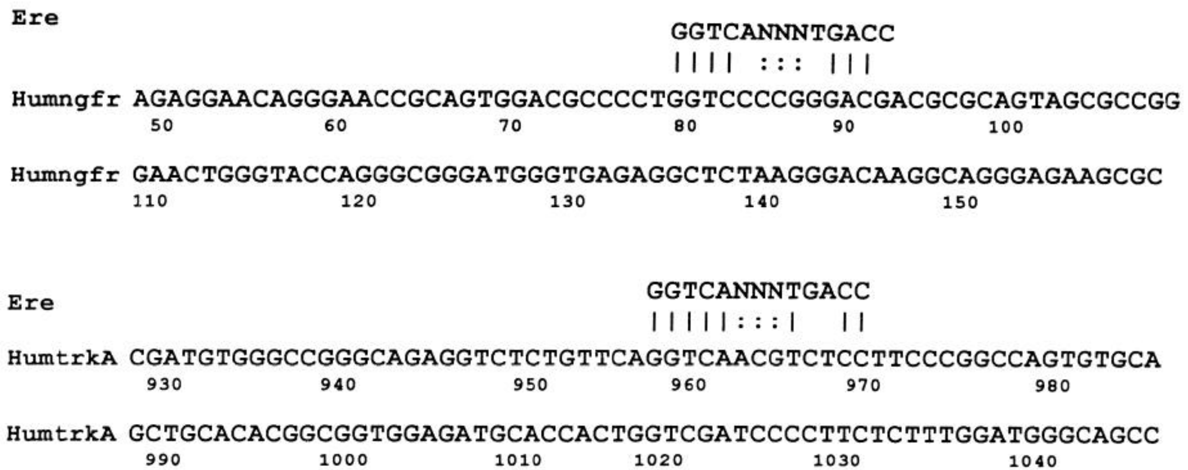

Figure 1. ERE-like sequences in p75^{NGFR} and *trkA* genes. Computer-assisted comparison of the 13 base palindromic ERE sequence (described for vitellogenin and c-*fos* genes) and the NGF receptor genes revealed close homology to sequences in the promoter region of the p75^{NGFR} gene and the 5′ region flanking the *trk* oncogene breakpoint in the *trkA* gene. Homologous nucleotides are indicated by *vertical bars*. *Humngfr*, human p75^{NGFR} sequence; *HumtrkA*, human *trkA* sequence.

A Uterus DRG
28S-
18S-
B Cortex P6 P8 P14
28S-
18S-
C Ctx P4 DRG Ctx P6
28S-
18S-

Figure 2. Specificity of oligonucleotide probes. Specificity of the oligonucleotide probes for the estrogen receptor, p75^{NGFR}, and *trkA* was determined by Northern blot analysis of total RNA. *A*, Hybridization of the estrogen receptor probe to a single (*arrowhead*) transcript (6.5 kb) in rat uterus and DRG RNA. *B*, Hybridization of the *trkA* oligonucleotide probe to a single (*arrowhead*) transcript (3.2 kb) in mouse cortex RNA, aged postnatal day (P) 6, P8, and P14. *C*, Hybridization of the p75^{NGFR} oligonucleotide probe to a single (*arrowhead*) transcript (3.8 kb) in P4, P6 mouse cortex and rat DRG RNA.

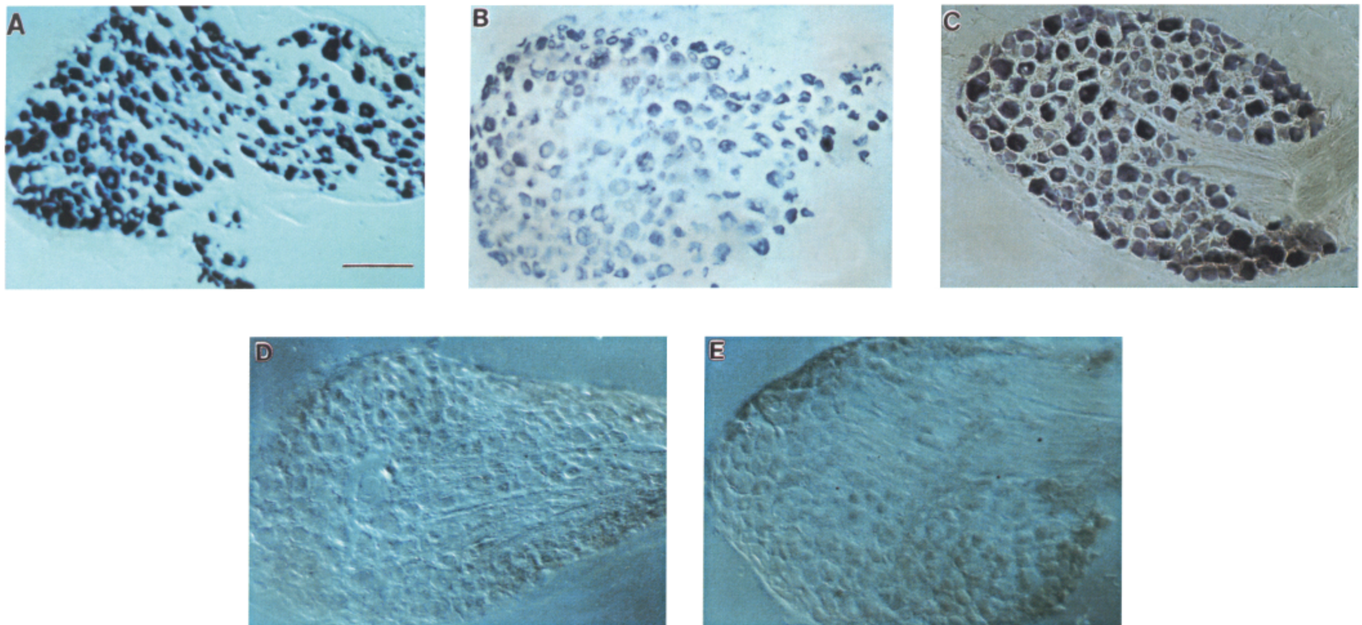

Figure 3. *A–E,* Estrogen receptor mRNA expression in DRG neurons. Twenty micrometer cryostat sections through DRG of intact and OVX females were hybridized to a 48 base digoxigenin-labeled oligonucleotide probe for the estrogen receptor. Note that the extent of hybridization to this probe was much greater in sensory neurons of the OVX female (*A*) than the intact female during proestrus (*B*) or the OVX female replaced with estrogen for 52 hr (*C*). *D,* Control for hybridization specificity; DRG sections (from OVX animals) incubated with the estrogen receptor probe *after* prior incubation with a 90 base oligonucleotide, overlapping the 48 base experimental probe, were always unstained. *E,* Immunohistochemical control; DRG sections from OVX animals hybridized with the noncomplementary (sense) oligonucleotide were also unstained. *A, C, D,* and *E* were photographed with Nomarski optics; *B,* bright-field photograph. Scale bar, 100 μm.

rotrophic factor, and neurotrophin-3. Neurotrophin signal transduction, however, appears to require the neurotrophins to associate with members of the tyrosine kinase receptor family of proto-oncogene products, *trkA* ($gp140^{trk}$) (Kaplan et al., 1991a,b) and the structurally related genes *trkB* (Soppet et al., 1991; Squinto et al., 1991) and *trkC* (Lamballe et al., 1991).

We have recently shown that estrogen receptor systems colocalize with $p75^{NGFR}$ mRNA and protein and *trkA* mRNA in neurons of the developing rodent medial septum, vertical and horizontal limbs of the nuclei of the diagonal band, substantia innominata, and nucleus basalis of Meynert (Toran-Allerand et al., 1992a; Miranda et al., 1993), as well as the cerebral cortex, striatum (caudate/putamen), and hippocampus (Miranda et al., 1993), regions previously described as targets of both NGF (Taniuchi et al., 1986; Yan and Johnson, 1987, 1988; Eckenstein, 1988; Koh and Loy, 1989; Mobley et al., 1989) and estrogen (Loy et al., 1988; Toran-Allerand et al., 1992b). The widespread colocalization of $p75^{NGFR}$ with estrogen-binding sites suggested that the central and peripheral targets of the neurotrophins may respond to estrogen as a general phenotypic characteristic. To address this issue we investigated the presence of estrogen receptor mRNA and estrogen-binding sites in a prototypical peripheral target of NGF, the dorsal root (sensory) ganglion (DRG) neuron of the adult female rat. DRG neurons are dependent on NGF for their survival during development (Barde, 1980; Johnson et al., 1980; Thoenen and Barde, 1980; Eichler and Rich, 1989) but only following injury in adulthood (Csillick, 1987; Lindsay, 1988; Rich et al., 1989). Neither estrogen receptor protein nor its mRNA has previously been demonstrated in DRG neurons, or indeed in other peripheral ganglia, although neurons of the superior cervical ganglia (SCG; Wright and Smollen, 1983a,b), hypogastric ganglion, and the major pelvic ganglia (Melvin and Hamill, 1986, 1989) have been reported to be sensitive to gonadal steroids.

We also addressed the general question of whether colocalization of estrogen and neurotrophin receptor mRNAs implies the potential for regulatory interactions, by examining whether estrogen responsiveness in DRG neurons would be manifested functionally as an effect of this gonadal hormone on neurotrophin receptor gene expression. In the present study, we first compared NGF receptor mRNA expression in DRG of ovariectomized (OVX; estrogen deficient) and intact animals at proestrus (physiologically high estrogen levels) and found that not only are estrogen receptor mRNA and estrogen-binding sites present in adult female rat DRG neurons but that the expression of the mRNAs for the estrogen receptor and the two NGF receptors $p75^{NGFR}$ and *trkA* is differentially modulated by the ovarian status of the animal. To assess directly the action of estrogen itself on NGF receptors mRNAs, we further compared OVX animals with those replaced with estrogen levels comparable to the morning of proestrus, and found a time- and receptor-specific regulation of NGF receptor mRNAs by estrogen. Our data support the hypothesis that the actions of estrogen on some target genes may involve intermediate steps, by modulating other transcription-regulating factors such as the neurotrophin receptors.

Materials and Methods

Tissue samples

Dorsal root ganglia were obtained from intact, OVX, and estrogen-treated OVX adult female rats (Sprague–Dawley, Zivic-Miller, Allison Park, PA). All animals were maintained in a 14 hr:10 hr light/dark cycle and given food and water ad libitum.

Proestrus/OVX study. The estrus cycle of intact females ($n = 8$) was monitored for at least 2 weeks (3–4 cycles) before use and animals were

killed between 1:00 and 2:00 P.M. on the day of proestrus (determined by vaginal smears). Bilaterally ovariectomized females were housed for 10 d before death and handled daily.

Estrogen replacement study. Bilaterally OVX animals purchased from Zivic-Miller (Allison Park, PA) were housed for 10 d before the experiment. They were then injected, subcutaneously, with 10 μg of estradiol benzoate in sesame oil (10:00 A.M., day 1), and killed 4 hr (2:00 P.M., day 1) or 52 hr later (2:00 P.M., day 3). Controls were injected with vehicle (sesame oil) only.

For *in situ* hybridization histochemistry, animals (n = 5/group) were anesthetized with pentobarbital (0.5 ml/100 gm) and perfused transcardially with isotonic saline, followed by 4% paraformaldehyde, containing 2.5% dimethyl sulfoxide (Sigma) and 0.1% diethyl-pyrocarbonate (Sigma), an RNase inhibitor. Cervical and lumbar ganglia were removed and postfixed for 2 hr in the same fixative. Tissue was allowed to sucrose-equilibrate overnight [15% sucrose in phosphate-buffered saline (PBS)] and embedded in M1 embedding matrix (Lipshaw). Cryostat sections (20 μm) were thaw mounted onto gelatin/polylysine-coated slides and processed immediately for *in situ* hybridization histochemistry. For RNA preparation, animals (OVX/proestrus, n = 3/group; estrogen replacement, n = 5/group) were anesthetized (as before), the spinal column exposed, and cervical and lumbar DRGs gathered immediately into liquid nitrogen. DRGs were stored at −70°C until use.

Preparation of oligonucleotide probes

Complementary ("antisense") and noncomplementary ("sense") oligonucleotide sequences were synthesized on an Applied Biosystems 380B DNA Synthesizer (Protein Core, Columbia University/Howard Hughes Medical Institute).

Estrogen receptor. A previously described (Miranda and Toran-Allerand, 1992) 48 base oligonucleotide sequence was used, corresponding to nucleotides 1923–1970 in the ligand-binding domain of the uterine estrogen receptor (Koike et al., 1987) with less than 25% homology with the other members of the steroid receptor superfamily of DNA-binding proteins.

NGF receptors. (1) For p75^{NGFR}, a previously reported (Ernfors et al., 1988; Toran-Allerand et al., 1992a), 46 base sequence from the putative membrane-spanning domain of the sequenced receptor in the chick, corresponding to nucleotides 807–852 of the rat homolog (Radeke et al., 1987) was used. (2) For *trkA*, a unique 60 base oligonucleotide sequence directed against nucleotides 208–267 in the putative extracellular domain of the human cDNA sequence (Martin-Zanca et al., 1989), corresponding to a region of the mouse *trkA* first exon (Martin Zanca et al., 1990), was used to detect *trkA* mRNA.

Complementary and noncomplementary oligonucleotide probes were 3′ end-labeled with digoxigenin-deoxyuridine-triphosphate (dig-dUTP; an average of 4 molecules/tail), using terminal deoxynucleotidyl transferase (Bethesda Research Labs) as previously described (Miranda and Toran-Allerand, 1992; Toran-Allerand and Miranda, 1992). Labeled probes were purified by sodium acetate/ethanol precipitation and diluted with TES buffer (10 mM Tris-HCl, 1 mM EDTA, 0.1% SDS, pH 8.0).

In situ *hybridization histochemistry*

In situ hybridization was performed according to the procedure described in Miranda and Toran-Allerand (1992). Briefly, sections were dehydrated in increasing ethanol grades, rehydrated without drying and rinsed for 5 min in 1× SSC (0.15 M NaCl, 0.015 M sodium citrate, pH 7.2). Sections were prehybridized in a buffer containing 50% formamide, 1 M NaCl, 1× Denhardt's solution, 10 mM dithiothreitol (in 0.01 M sodium acetate, pH 5.2), and 0.5 mg/ml tRNA for 2 hr at room temperature. Sections were then incubated 16–18 hr with the oligonucleotide probe (diluted 20 ng/ml in the above buffer) at 42°C, rinsed twice in 1× SSC, washed for 2 hr in either 0.2× SSC at 42°C (estrogen receptor and p75^{NGFR} probes) or in 0.1× SSC at 60°C (*trkA* probe), and washed overnight (42°C) in 1× SSC. Tissue sections were then blocked for 30 min with 5% nonfat dry milk (Carnation) in Tris-buffered saline (TBS; 10 mM Tris, 140 mM NaCl, pH 7.4) containing 0.3% Triton X-100 and then incubated in a 1:500 dilution of the polyclonal sheep anti-digoxigenin antibody, Fab fragment, conjugated to alkaline phosphatase (Boehringer Mannheim) prepared in the blocking solution, for 48 hr at 4°C. Sections were washed three times for 15 min in TBS and soaked for 5 min in buffer A (100 nM Tris, 200 mM NaCl, and 50 mM $MgCl_2$, pH 9.5). Hybrids were visualized with a color reaction catalyzed by antibody-conjugated alkaline phosphatase, using nitroblue tetrazolium salt (0.225 μg/ml) and 5-bromo-4-chloro-3-indolyl-phosphate (0.175 μg/ml) as substrates in buffer A, with 0.24 mg/ml levamisole to inhibit endogenous alkaline phosphatase activity. The color of the alkaline phosphatase reaction product depends critically on the pH of the substrate buffer (A), and can vary from a blue-purple (pH 9.5) to a brown-black (pH 7.5-8.0) coloration. For any given probe, one animal each from the following comparison groups was run concurrently, that is, proestrus/OVX or OVX + vehicle/OVX + 4hrE/OVX + 52hrE. Color development was closely monitored, and the reaction was terminated for all groups (5 min in 10 mM Tris + 1 mM EDTA), when color appeared fully developed in one condition. Slides were dehydrated briefly through ethanol grades, cleared in Histoclear (National Diagnostics), and coverslipped in Permount. Hybridization and immunohistochemical controls for the estrogen receptor oligonucleotide are described in Results. Extensive controls, ensuring the specificity of the nonisotopic *in situ* hybridization method and the probes used here, are described in Miranda and Toran-Allerand (1992) and Toran-Allerand and Miranda (1993).

Hybridization specificity of oligonucleotide probes

Hybridization specificity of each oligonucleotide probe was characterized by Northern blot analysis. Total RNA, prepared by a modification (Lu et al., 1989) of the Chomczynski and Sacchi (1986) method, was size fractionated on a 1.2% agarose denaturing gel and capillary transferred to nylon membrane (Hybond-N, Amersham). Filters were prehybridized overnight at 42°C in 4× SSPE, 40% formamide, 5% dextran sulfate, 25 μg/ml salmon sperm DNA, 100 μg/ml tRNA, 0.04× Denhardt's solution, and 0.2% SDS. The probes were 5′ end-labeled with γ-^{32}P-ATP, using T4-polynucleotide kinase, and hybridization was performed at 42°C for 24 hr. Washes were performed under conditions of gradually increasing stringency (4× SSC to 0.2× SSC, and from room temperature to 50°C). Nylon membranes were apposed to Kodak X-Omat film between intensifying screens at −80°C for 1–4 d.

Northern analysis of total rat uterine RNA probed with the estrogen receptor oligonucleotide probe revealed hybridization to a single transcript (Fig. 2*A*) of the previously reported size [6.5 kilobases (kb) Koike et al., 1987]. A similar-sized transcript was seen in RNA obtained from OVX rat DRG. The 46 base p75^{NGFR} oligonucleotide sequence was identical to that reported by Ernfors et al. (1988), and Northern blot analysis of DRG-derived mRNA probed with this oligonucleotide revealed hybridization to the expected single transcript, approximately 3.8 kb in size (Fig. 2*C*). Total RNA probed with the oligonucleotide probe for *trkA* showed a single band of hybridization (Fig. 2*B*) to a 3.2 kb transcript (Martin-Zanca et al., 1989).

Northern blot analysis

Total RNA was size fractionated (10–25 μg/lane) on a 1.2% agarose, 18% formaldehyde gel and capillary transferred to nylon membrane (Hybond-N, Amersham). Filters were prehybridized (4–18 hr) and hybridized (36–40 hr) at 37°C in 50% formamide, 3× SSC, 0.1 M Tris, 5× Denhardt's, and 10% dextran sulfate. Probes were labeled with α-^{32}P-dCTP, using a random priming kit (Boehringer Mannheim). *TrkA* mRNA was identified by hybridization with pDM97, a 450 base pair (bp) *trkA*-specific probe corresponding to the extracellular and transmembrane domains of the *trkA* gene (Martin-Zanca et al., 1989; gift of L. F. Parada). p75^{NGFR} was probed by hybridization with p5b, a 2 kb probe corresponding to the extracellular domain of the p75^{NGFR} (Buck et al., 1988). Blots were washed in 0.2× SSC and 0.5% SDS, briefly at room temperature first and then for 2 hr at 60°C, with frequent changes. Filters were then exposed to film (Kodak X-Omat) between intensifying screens and stored at −70°C for 1–4 d before developing.

Using a standard morphometrics package (Jandel Scientific), optical density measurements were obtained from x-ray film. *trkA* and p75^{NGFR} transcript densities were normalized to an internal control to account for variation in the amount of RNA loaded. Since estrogen has been reported to enhance the expression of commonly used controls such as β-actin (Guo and Gorski, 1988) and 28S (Jones et al., 1986), but not 18S, ribosomal RNA (Yu et al., 1991), NGF receptor transcripts in this study were normalized to 18S ribosomal RNA bands, visualized by ethidium bromide or by a riboprobe specific for 18S (Ambion, Austin, TX). Group differences for the proestrus/OVX experiments were evaluated using a t test while group differences for the estrogen replacement study were evaluated using a one-way ANOVA, with Student-Newman-Keuls test of planned post hoc comparisons. Differences were considered significant at the 0.05 level.

Estrogen receptor binding assay

Estrogen-binding sites were identified by a modification of the nuclear exchange assay (MacLusky et al., 1990). Ten days after surgery, bilaterally ovariectomized and adrenalectomized rats (Sprague–Dawley, Charles River, Kingston, NY) were deeply anesthetized (pentobarbital, 0.5 ml/100 gm) and their (cervical/lumbar) DRGs removed and collected in ice-cold medium [Dulbecco's minimum essential medium (MEM), 0.65% glucose, 25% gelded horse serum). Ganglia were crudely freed of meningeal tissue and basement membrane and incubated with a 2 nM concentration of the synthetic estrogen 11β-methoxy-^{3}H-moxestrol (R2858; Du Pont–New England Nuclear; specific activity, 80 Ci/mmol; $n = 3$), for 2 hr at 37°C ($n = 3$). For some animals ($n = 2$), DRG were incubated with 2 nM 16α-^{125}I-iodo-3,17β-estradiol (specific activity, 2200 Ci/mmol; Du Pont–New England Nuclear). In all cases, half the ganglia harvested were incubated with the radioligand in the presence of 2 μM unlabeled estrogen (17β-estradiol or diethylstilbestrol, DES). After incubation, DRG were washed three times in ice-cold MEM + 0.1% BSA, for 5 min each, transferred to a homogenizer (clearance, 0.125 mm), and homogenized in 300 μl of a 1 mM potassium phosphate, 3 mM magnesium chloride, and 0.25% Triton X-100 (pH 6.5) solution. The homogenate was centrifuged briefly to recover a pellet and washed twice in the same buffer without Triton X-100. Pellets were then eluted with 1 ml of 95% ethanol (estrogen is fully soluble in >50% ethanol), and the radioactivity counted in a liquid scintillation counter. Pellets were dried and resuspended in 1× TE (10 mM Tris, 1 mM EDTA) and assayed fluorometrically for DNA content, using the Hoechst dye 33258 (Cesarone et al., 1979). Specific binding was determined as the difference between total binding and nonspecific binding (in the presence of an unlabeled competing ligand), and the data are expressed as femtomoles of specifically bound ligand/mg DNA.

To demonstrate specificity of estrogen binding sites in DRG neurons, additional experiments were performed using four groups of DRG. DRG were harvested as before and incubated in phenol red–free RPMI 1640 media supplemented with 25% gelded horse serum with ^{3}H-moxestrol alone or ^{3}H-moxestrol and 2 μM DES, or the nonestrogenic hormones dihydrotestosterone (DHT) or progesterone. All subsequent analyses were as described before.

Estrogen assay

Circulating estrogen levels were assayed from the plasma of proestrus and untreated/estrogen-treated OVX animals. Blood was obtained from the internal carotids of deeply anesthetized (pentobarbital, 0.5 ml/100 gm) animals and collected in ice-cold, heparin-coated vials. Samples were then spun on a clinical centrifuge for 10 min, and the supernatant (plasma) stored at −20°C until assayed. Estrogen content was determined by a solid-phase ^{125}I radioimmunoassay (Diagnostic Products, Los Angeles, CA).

Results

Estrogen receptor mRNA and estrogen binding sites are present in adult DRG

Estrogen receptor mRNA expression was found in virtually all DRG neurons of the intact and OVX animal, using nonisotopic (digoxigenin) *in situ* hybridization histochemistry with an oligonucleotide probe (Miranda and Toran-Allerand, 1992) to the ligand-binding domain of the rat uterine estrogen receptor. The staining intensity of the hybridization product appeared strongest in ganglion neurons obtained from OVX rats. As seen in Figure 3*A*, all large and small neurons in the ganglion hybridized the probe, as evidenced by the dark blue/purple cytoplasmic reaction product. Nuclei generally remained unstained. However, although hybridization was characteristically restricted to the cytoplasm, alkaline phosphatase results in a flocculent reaction product, and where the probe hybridized abundantly, as in this instance, overstaining makes the nucleus less readily apparent. Non-neuronal cells, such as satellite cells and fibroblasts, did not hybridize the estrogen receptor probe, although occasional hybridization was seen in Schwann cells, and there was no hybridization signal in neuropil and axon bundles.

The estrogen receptor probe used here is highly specific for the estrogen receptor (Miranda and Toran-Allerand, 1992) and, by Northern blot analysis, recognizes a band of approximately 6.5 kb (Koike et al., 1987) in rat uterine RNA and DRG RNA from OVX rats (Fig. 2*A*). To ensure that the reaction product was the result of specific hybridization to the oligonucleotide, some sections were incubated with the estrogen receptor probe *after* prior incubation in a 100-fold excess of an unlabeled, 90 base oligonucleotide (deduced amino acids 564–593) that completely overlapped the 48 base experimental probe. These sections always remained unstained (Fig. 3*D*). As an immunohistochemical control, some sections from each DRG were hybridized with a noncomplementary ("sense") oligonucleotide and, as seen in the representative example in Figure 3*E*, these sections always remained unstained, indicating a lack of hybridization.

A modification of the nuclear exchange assay (MacLusky et al., 1990; see Materials and Methods) was used to determine the presence of estrogen-binding sites in sensory ganglia. In these experiments, DRG were obtained from ovariectomized and adrenalectomized rats to minimize receptor occupancy by any endogenous estrogen. Similar levels of competable binding were seen using either 16α-^{125}I-iodo-3,17β-estradiol (31.36 fmol/mg DNA) or 11β-methoxy-^{3}H-moxestrol (39.43 fmol/mg DNA), with a mean specific nuclear binding of 34.5 (±8.58) fmol/mg DNA. The nonestrogenic ligands DHT and progesterone were not able to compete moxestrol binding, suggesting that binding was specific to the estrogen receptor. Since estrogen receptor mRNA localized primarily to sensory neurons, specific binding in these ganglia suggests that estrogen receptor mRNA is translated into its encoded protein in these neurons.

Ovarian hormones downregulate estrogen receptor mRNA in DRG neurons

Not only was estrogen receptor mRNA present in all DRG neurons, but the extent of hybridization to the estrogen receptor probe appeared to be regulated by the estrogen status of the animal. In the OVX rat (Fig. 3*A*) the pattern of hybridization (staining) was virtually homogeneous within a ganglion. Thus, in the estrogen-deficient state, every DRG neuron appeared to express estrogen receptor mRNA strongly. In plasma obtained from OVX animals 11 d after gonadectomy, estrogen levels were below sensitivity of the assay (<8 pg/ml; see Fig. 4). In contrast, on the afternoon of proestrus, soon after estrogen levels peak physiologically and shortly before the estrus-related decline in estrogen, the pattern of hybridization to the estrogen receptor probe was more heterogeneous, as compared to the OVX condition (Fig. 3*B*). While there were a few darkly stained neurons present, the hybridization signal was markedly reduced in the majority of neurons in this group. Plasma estrogen levels for this group were on average 30 pg/ml, consistent with expected estrogen levels on the afternoon of proestrus (Butcher et al., 1974). Hybridization to estrogen receptor mRNA also appeared markedly reduced in OVX animals, 52 hr after a single dose of estrogen (Fig. 3*C*). While some neurons strongly hybridized the probe, in most instances the hybridization signal in DRG of estrogen-replaced OVX animals at 52 hr was markedly reduced or virtually absent, although this effect was variable at 4 hr after estrogen treatment (data not shown). Plasma estrogen levels at 4 hr after a single injection of estrogen (Fig. 4) were high, resembling levels typically seen on the morning of proestrus, while at 52 hr, estrogen levels had fallen almost threefold. It should

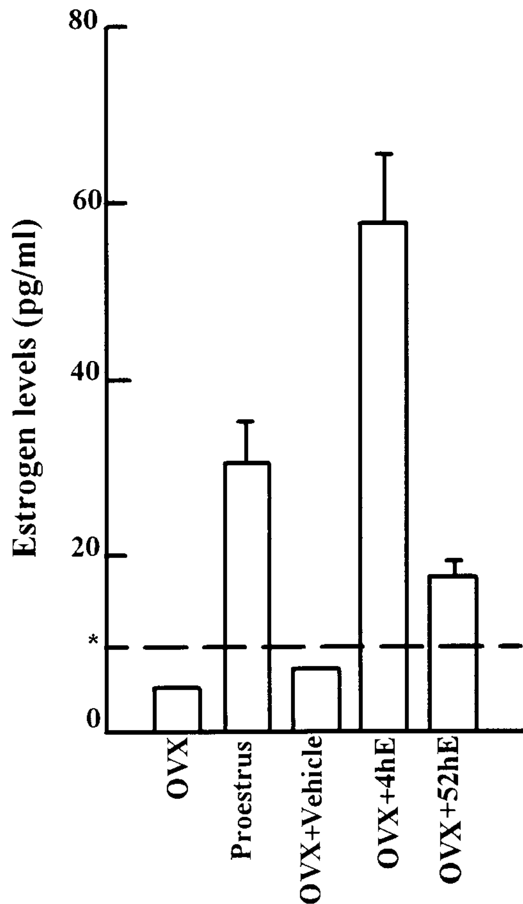

Figure 4. Plasma estrogen levels. Plasma estrogen levels were determined in OVX, proestrus, and estrogen-replaced OVX animals using an iodinated estradiol antibody kit. Bar graph represents the mean (±SEM) estrogen levels detected, consisting of three animals from each hormonal condition. The level of estrogen in untreated (*OVX*) and vehicle-treated OVX (*OVX+ Vehicle*) animals was undetectable by this assay (below 8 pg/ml) while the mean estrogen levels of intact (*Proestrus*) animals agrees closely with those reported the afternoon of proestrus (Butcher et al., 1974). OVX animals given a single injection of EB (10 μg) had high levels of estrogen 4 hr later (*OVX+4hE*), comparable to those seen on the morning of proestrus, which declined threefold when measured 52 hr (*OVX+52hE*) after the injection. *Asterisk* indicates 8 pg/ml, the lower limit of the sensitivity of the assay.

be noted that in the intact animal, estrogen titers that are very low at metestrus, rise gradually to peak on the morning of proestrus (2–2.5 d later) and fall shortly thereafter. Hence, the decrease in estrogen receptor mRNA hybridization during proestrus and at 52 hr after estrogen treatment suggests, as has been shown in the brain (Lauber et al., 1990; Simerly and Young, 1991; Shughrue et al., 1992), that prolonged exposure to estrogen downregulates the expression of its own receptor mRNA in sensory neurons.

Ovarian hormones upregulate NGF receptor mRNAs

We also analyzed the expression of the two NGF receptor mRNAs $p75^{NGFR}$ and *trkA* in OVX, proestrus, and estrogen-replaced OVX animals, by *in situ* hybridization histochemistry, using oligodeoxynucleotide probes, and by Northern blot analysis, using cDNA probes.

Regulation of $P75^{NGFR}$ *mRNA.* As seen in Figure 5, *A* and *B*, DRG neurons, hybridized with the oligonucleotide probe for $p75^{NGFR}$ mRNA, exhibited some level of expression for this receptor mRNA irrespective of the hormonal status of the animal. While both large and small neurons were equally likely to express $p75^{NGFR}$ mRNA, there was a marked difference in the pattern of hybridization to the $p75^{NGFR}$ probe, depending upon the estrogen status of the animal. While some DRG neurons from OVX animals did hybridize the $p75^{NGFR}$ probe, Figure 5*A* shows that there were many neurons present that did not detectably express this mRNA. The proportion of DRG neurons, from OVX animals, expressing $p75^{NGFR}$ mRNA varied from one experimental run to another; hence, their numbers were not quantified, although these ranged approximately from one-fourth to one-half of the neurons present in a section. Among those neurons that did express the mRNA, a few were darkly stained but the majority had a pale hybridization product, indicating that the expression of hybrids in these neurons was significantly low. This pattern of hybridization was in striking contrast to the one seen in DRG neurons of the proestrus animal probed for $p75^{NGFR}$ mRNA. In these ganglia, virtually every neuron appeared to express the mRNA, and the homogeneous dark blue-black hybridization product (Fig. 5*B*) indicated that the expression of this mRNA within a given neuron was much higher on average in proestrus than OVX animals.

Northern blot analysis (Fig. 6*A*) of total RNA from OVX and proestrus DRGs confirmed the differences observed in the *in situ* hybridization analysis. Optical density measurements of the $p75^{NGFR}$ transcript (normalized to 18S ribosomal RNA) indicated a 62% ($p < 0.05$) increase in $p75^{NGFR}$ mRNA during proestrus, as compared to OVX controls. The Northern analysis confirmed the direction of proestrus induced changes seen by *in situ* hybridization analysis, although the colorimetric reaction appears to exaggerate the difference in $p75^{NGFR}$ mRNA expression. However, the alkaline phosphatase reaction is not linear and therefore unsuitable for quantitation, unlike the Northern blot analysis. Our results thus indicate that the hormonal conditions existing at proestrus thus appeared to *upregulate* NGF receptor mRNA, in contrast to the *downregulation* of estrogen receptor mRNA.

Regulation of trkA *mRNA.* Hybridization with the oligonucleotide probe for *trkA* mRNA in DRG neurons revealed an expression pattern similar to that of $p75^{NGFR}$ mRNA, that is, a reduced level of expression in OVX animals, as compared to proestrus. In the OVX animal (Fig. 5*C*), a few darkly labeled neurons were generally found scattered in the periphery and center of the ganglion. The majority of neurons, however, were pale colored, indicative of significantly lowered levels of mRNA expression. However, the pattern of hybridization was considerably altered during proestrus, where almost every neuron in the ganglion appeared to hybridize strongly with the *trkA* probe, as shown by the dark blue-black staining of the DRG neurons of Figure 5*D*. Both the number of hybridizing neurons and the intensity of the hybridization signal appeared greater in the proestrus condition.

Northern blot analysis of *trkA* RNA obtained from DRGs of proestrus and OVX animals confirmed the observed differences in the pattern of hybridization seen by *in situ* hybridization (Fig. 6*B*). The levels of *trkA* mRNA, measured densitometrically and normalized to an internal control (18S ribosomal RNA), were, on average, threefold (285%) greater during proestrus as compared to the OVX condition. Thus, both NGF receptor mRNAs

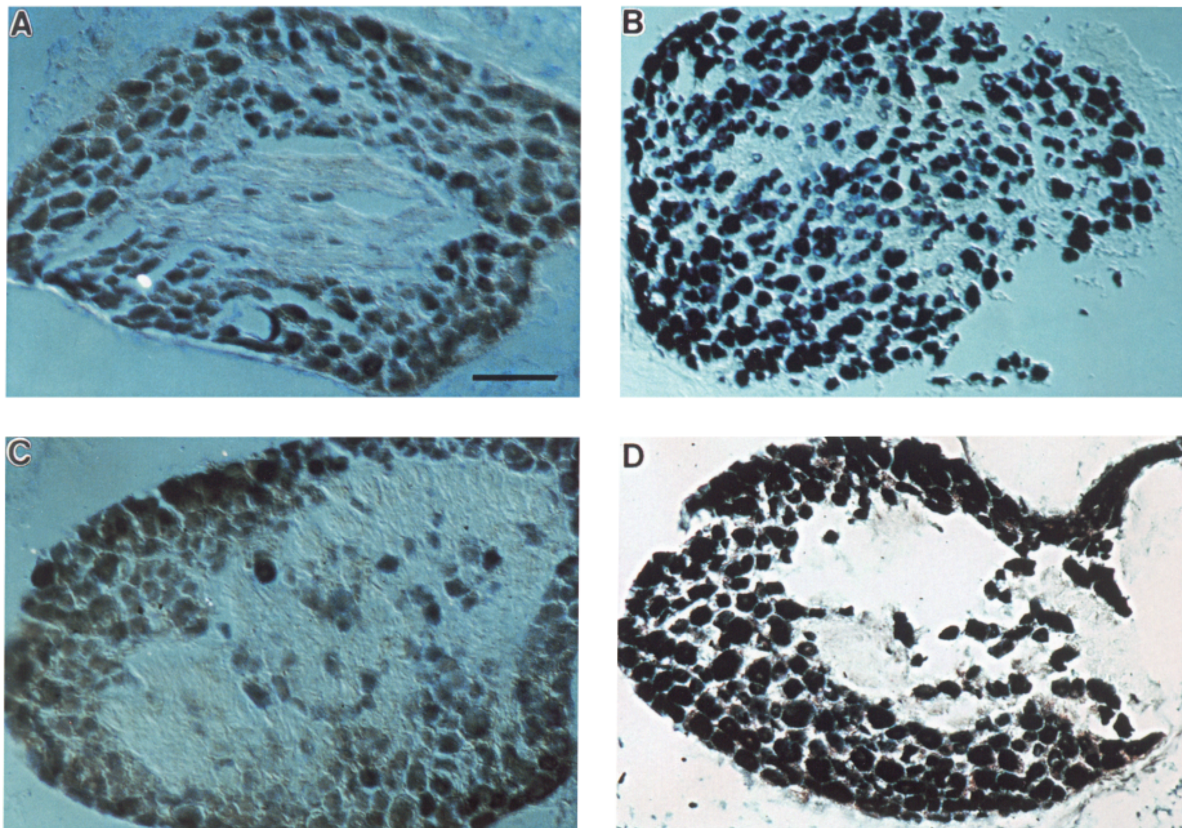

Figure 5. *A–D*, NGF receptor mRNAs in DRG neurons. Twenty micrometer cryostat sections through DRG of OVX and intact females were hybridized to digoxigenin-labeled oligonucleotide probes for the $p75^{NGFR}$ (46 bases) and *trkA* (60 bases) mRNA. While NGF receptor mRNAs were expressed in both hormonal conditions, the extent of hybridization to $p75^{NGFR}$ (*upper panels*) and *trkA* (*lower panels*) mRNA was much less in OVX (*A* and *C*) animals as compared to intact animals during proestrus (*B* and *D*). (*A–C* were photographed with Nomarski optics; *D*, bright-field photograph.) Scale bar, 100 μm.

appeared to be increased in the proestrus condition, when compared to the OVX animals, *trkA* more so than $p75^{NGFR}$.

Estrogen regulation of $p75^{NGFR}$ and trkA *mRNA*

To assess directly the effect of estrogen on NGF receptor mRNA, we then compared OVX animals with those replaced with a single dose of estrogen (estradiol benzoate, EB) and killed 4 or 52 hr later. Plasma estrogen assays indicated that, while OVX animals had undetectable levels of estrogen, 4 hr after a single dose of estrogen, estrogen titers rose to 58 pg/ml, comparable to levels seen in the morning of proestrus. Fifty-two hours after a single injection of estrogen, estrogen levels had fallen to 17 pg/ml (Fig. 4).

Northern blot analysis of $p75^{NGFR}$ mRNA from DRGs obtained from animals replaced with estrogen indicated that estrogen transiently downregulated this receptor mRNA ($p < 0.05$; Fig. 7*A*). Levels of $p75^{NGFR}$ measured densitometrically (normalized to 18S ribosomal RNA) were reduced by half, 4 hr after a single dose of EB and were no different in OVX animals killed 52 hr after a single injection of estrogen, as compared to vehicle-treated controls.

However, in the case of *trkA* (Fig. 7*B*), in contrast, densitometric measurements of this transcript in Northern blot analysis indicated that estrogen treatment resulted in a twofold increase in mRNA expression of this NGF receptor 4 hr after a single injection of estrogen ($p < 0.01$) and a threefold increase in *trkA* mRNA in OVX animals exposed to 52 hr of estrogen, as compared to vehicle treated controls ($p < 0.01$). The levels of *trkA* mRNA observed in the OVX + 4hrE group was not significantly different from the OVX + 52hrE group, indicating an early and prolonged upregulation of *trkA* mRNA by estrogen. Estrogen regulation of *trkA* mRNA was also detected by *in situ* hybridization analyses, using a digoxigenin-labeled oligonucleotide probe for *trkA*. Hybridization patterns of the *trkA* probe, *in situ* (Fig. 8*A*,*B*), closely paralleled Northern analyses of the *trkA* transcript (Fig. 7*B*), with respect to estrogen treatment. In general, the extent of hybridization of the *trkA* probe in DRG obtained from vehicle-treated controls appeared low. While there were a few darkly staining neurons, the majority of neurons were either unstained or had a pale blue color, indicating an overall low level of hybridization product in DRG neurons within this group (Fig. 8*A*). In contrast, hybridization product was seen in virtually every neuron of DRG obtained from OVX animals exposed to estrogen for 52 hr (Fig. 8*B*), with many darkly staining neurons present throughout the ganglion. The extent of hybridization to the *trk4* probe in DRG from OVX animals, 4 hr after estrogen treatment (data not shown), appeared intermediate to those seen in the OVX and OVX + 52hrE groups. $p75^{NGFR}$ mRNA regulation by estrogen was not amenable to *in situ* hybridization analyses given that the already low hybridization to the $p75^{NGFR}$ probe in DRG neurons of untreated OVX animals (example shown in Fig. 5*A*) would be further decreased in DRG neurons of estrogen-treated OVX animals (as indicated by Northern blot analyses), requiring de-

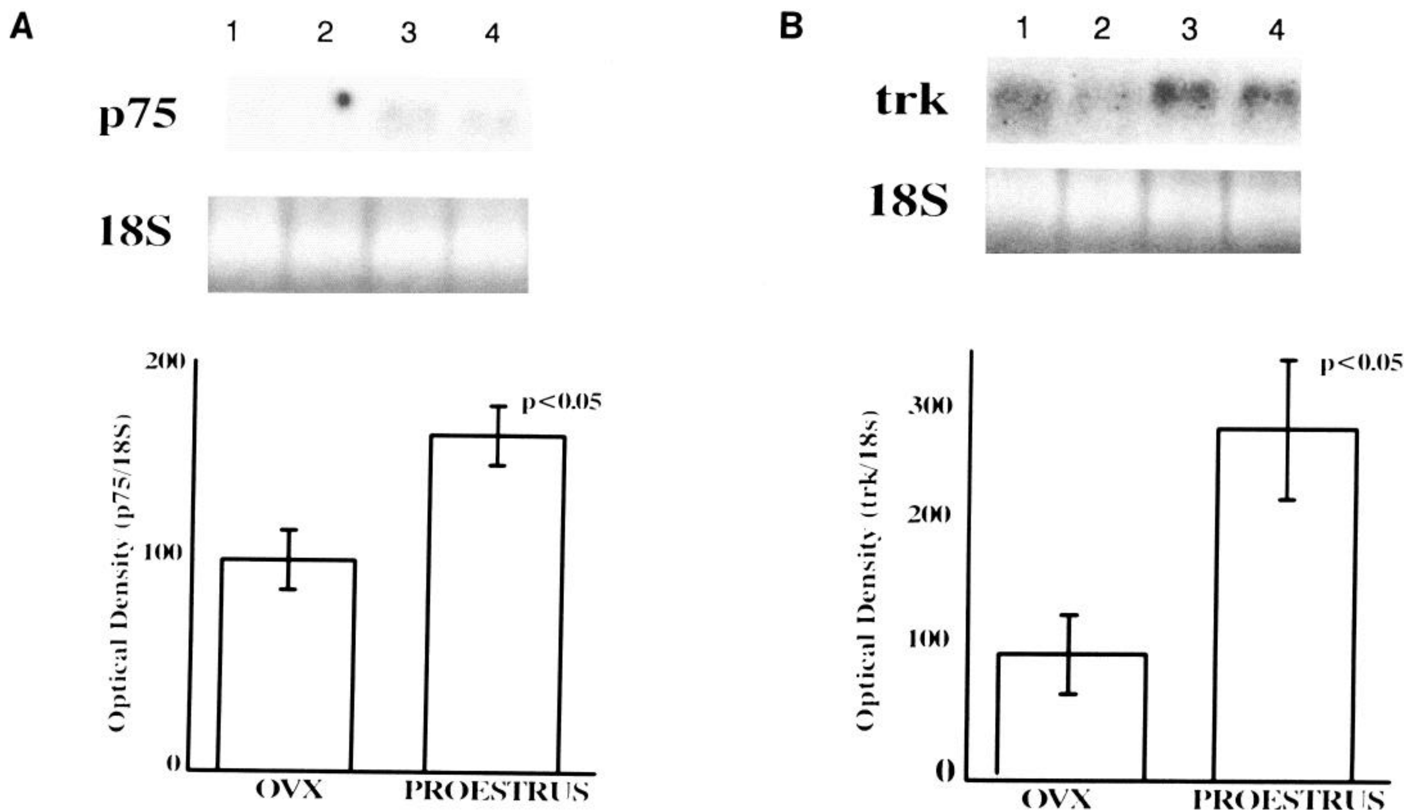

Figure 6. Northern blot analysis of DRG RNA. Total RNA, obtained from DRG of OVX (*lanes 1* and *2*) and proestrus (*lanes 3* and *4*) females, loaded 15 μg per lane, was hybridized to random-primed, ^{32}P-labeled cDNA probes for the NGF receptors. *A*, p75^{NGFR} was probed by hybridization with a 2 kb cDNA probe corresponding to the extracellular domain of the p75^{NGFR} gene (p5b; Buck et al., 1988). *B*, *trkA* mRNA was identified by hybridization with a 450 bp *trkA*-specific cDNA probe corresponding to the extracellular and transmembrane domains of the *trkA* gene (pDM97; Martin-Zanca et al., 1989; gift of L. F. Parada). To quantify group differences, optical density of the NGF receptor transcripts was normalized to 18S ribosomal RNA, to control for variations in the amount of total RNA loaded onto the gel. Bar graph represents mean (±SEM) of three animals from each hormonal condition. Statistically significant ($p < 0.05$) differences as compared to the untreated OVX group are indicated.

tection of differences beyond the sensitivity of most, including this, colorimetric assay.

Discussion

The present data indicate that adult female rat sensory neurons are estrogen targets, *in vivo,* and that estrogen treatment differentially modulates the expression of estrogen and NGF receptor mRNAs. These findings suggest that estrogen may regulate peripheral neuronal sensitivity to both estrogen and the neurotrophins. Our data indicate that estrogen receptor mRNA and specific, competable, nuclear estrogen-binding sites (receptors) are present in adult sensory ganglia. Moreover, the estrogen status of the animal appears to regulate reciprocally the expression of estrogen receptor mRNA and the mRNAs for the NGF receptors p75^{NGFR} and *trkA* in adult sensory neurons. While estrogen receptor mRNA is downregulated and NGF receptors upregulated during proestrus, estrogen replacement in OVX animals, in contrast, appears to downregulate mRNA for the estrogen receptor and the pan-neurotrophin receptor p75^{NGFR}, while upregulating *trkA* mRNA. The present findings are consistent with our hypothesis that estrogen sensitivity may be a general feature of the neural targets of neurotrophins, in this case NGF, and that some of estrogen's action on neural tissue may result via estrogen-induced transcriptional regulation of the neurotrophin receptors. The potential universality of these interactions is suggested, moreover, by preliminary findings that PC12 cells, another prototypical target of NGF, have estrogen-binding sites, and in this cell type as well, estrogen appears to regulate differentially both p75^{NGFR} and *trkA* mRNA (F. Sohrabji, L. A. Greene, R. C. Miranda, and C. D. Toran-Allerand, unpublished observations).

Although estrogen receptors have not been previously reported in adult sensory neurons, the widespread expression of estrogen receptor mRNA observed in DRG neurons and the presence of nuclear estrogen-binding sites argue that these neurons are estrogen targets. While the levels of estrogen-binding sites in sensory ganglia appear low, they are within the range reported for estrogen targets in the adult brain (Friedman et al., 1983). It should be noted, however, that the estimates of estrogen-binding sites reported here may underestimate the proportion of estrogen receptors in DRG neurons. First, binding sites were normalized to *total* DNA content of the ganglia, while the *in situ* hybridization analysis indicates that estrogen receptor mRNA was predominantly expressed only in neurons and not support cells of the DRG. Additionally, binding assays that used Dulbecco's MEM (see Materials and Methods) were not phenol red free, and a contaminant found intermittently in phenol red dye lots is reportedly estrogenic (Berthois et al., 1986; Bindal and Katzenellenbogen, 1988), which may contribute to underestimates of actual binding sites. In fact, preliminary assays using phenol red–free media suggest that estrogen binding estimates in DRG are substantially higher. In addition to the

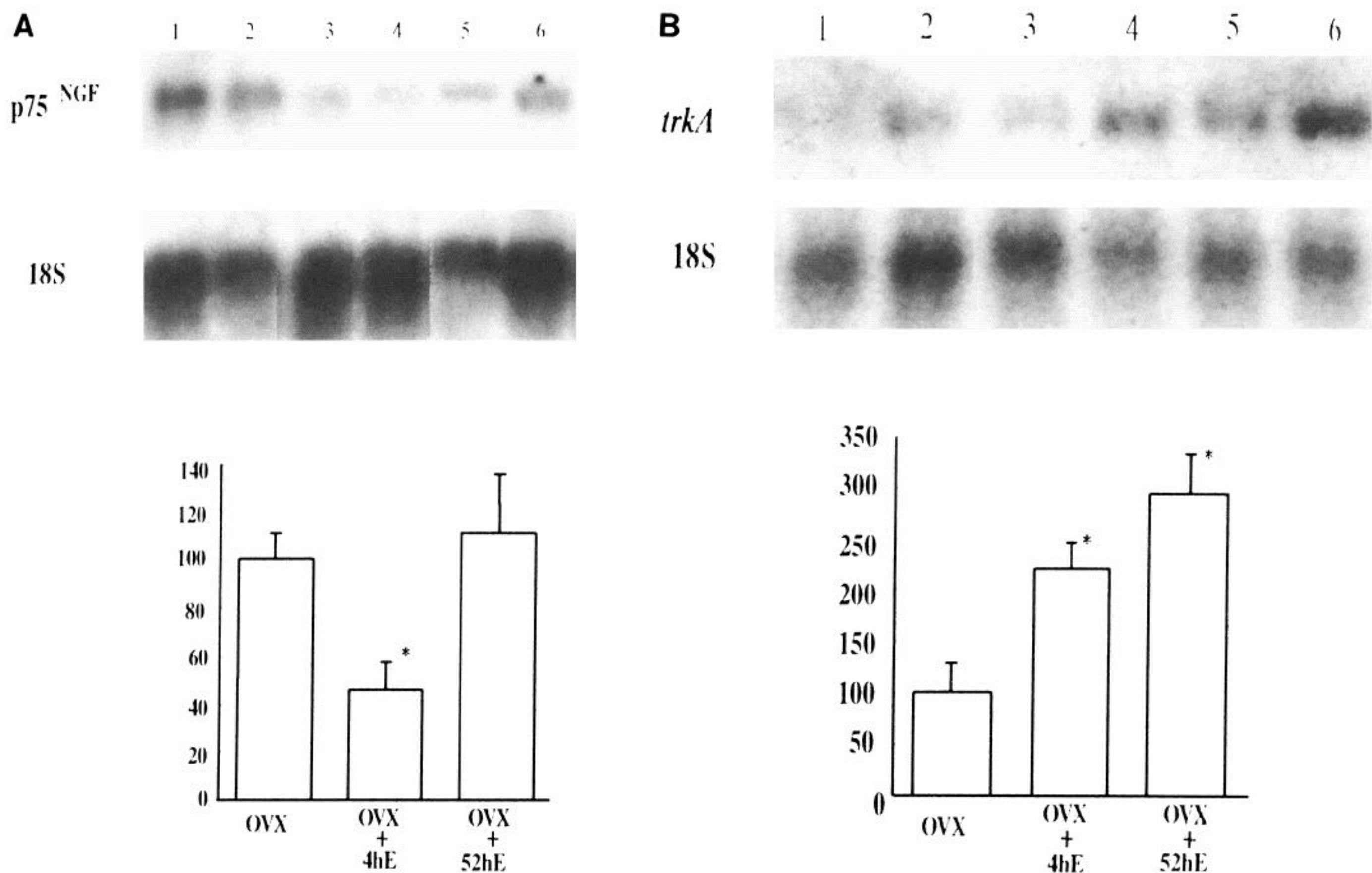

Figure 7. Northern blot analyses of estrogen regulation of NGF receptor mRNAs. Total RNA, loaded 25 μg per lane, was obtained from DRG of vehicle-treated OVX animals (*lanes 1* and *2*) and OVX animals killed 4 hr (*lanes 3* and *4*) and 52 hr (*lanes 5* and *6*) after a single injection of EB. Nylon filters were hybridized with random-primed, ^{32}P-labeled cDNA probes for either a 2 kb cDNA probe corresponding to the extracellular domain of the p75^{NGFR} gene (*A*) (p5b; Buck et al., 1988), or a 450 bp *trkA*-specific cDNA probe corresponding to the extracellular and transmembrane domains of the *trkA* gene (*B*) (pDM97; Martin-Zanca et al., 1989; gift of L. F. Parada). To quantify group differences, optical density of the NGF receptor transcripts was normalized to 18S ribosomal RNA, to control for variations in the amount of total RNA loaded onto the gel. Bar graph represents mean (±SEM) of five animals from each group. *Asterisks* indicate statistically significant ($p < 0.05$) differences as compared to the vehicle-treated OVX group.

binding assays, the effect of estrogen on NGF receptor mRNA expression further supports the concept of the existence of functional estrogen receptors in DRG neurons. Our present data are also consistent with reports that *developing* DRG and other peripheral neurons are responsive to estrogen. For example, neonatal exposure to estrogen reportedly increases both neuron and synapse number in the SCG (Wright and Smollen, 1983a). More recently, it has been shown that those lumbosacral spine motoneurons that innervate the bulbocavernosus muscle [spinal nucleus of the bulbocavernosus (SNB)], if exposed to early estrogen treatment, initiate elaborate dendrite arborizations (D. Sengelaub, personal communication). Since neither estrogen receptors (binding sites) (Pfaff and Keiner, 1973; Sar and Stumpf, 1977; Breedlove and Arnold, 1980) nor mRNA (Simerly et al., 1990) have been found in adult SNB motoneurons, this dendrite growth may well result indirectly from the action of estrogen on those neurons and processes afferent to the SNB, namely, the L5, L6, and S1 DRG neurons.

In the present study, the expression of estrogen receptor mRNA was markedly reduced in DRG neurons of the proestrus animal, as well as OVX animals 52 hr after exposure to estrogen, as compared to ovariectomized controls. Both the direction and relative time course of estrogen-dependent downregulation of estrogen receptor mRNA in sensory neurons are similar to that observed in the adult brain (Simerly and Young, 1991; Lauber et al., 1992) and extraneural targets of estrogen such as the uterus (Shupnik et al., 1989). In the case of proestrus animals, furthermore, downregulation of estrogen receptor mRNA in sensory neurons is similar to that observed in the intact adult brain compared to the OVX condition. For example, in established targets of estrogen such as the medial preoptic area and the ventromedial and arcuate nuclei of the hypothalamus, ovariectomy reportedly increases the level of estrogen receptor mRNA, when compared to any stage in the estrus cycle of the intact female (Shughrue et al., 1992). Similarly, OVX animals with hormone replacement also show marked downregulation of estrogen receptor mRNA in the ventrolateral aspect of the ventromedial nucleus and in the arcuate nucleus of the hypothalamus 18 hr and later, although this effect is not apparent at 4 hr following estrogen treatment (Lauber et al., 1992). Exposure to estrogen in the form of an implanted silastic capsule also depressed estrogen mRNA levels in these regions, when measured 24 and 72 hr later (Simerly and Young, 1991). In the present study, while estrogen consistently downregulated its receptor mRNA at 52 hr, the effects of estrogen at 4 hr were more variable, being downregulated in some cases, and unchanged

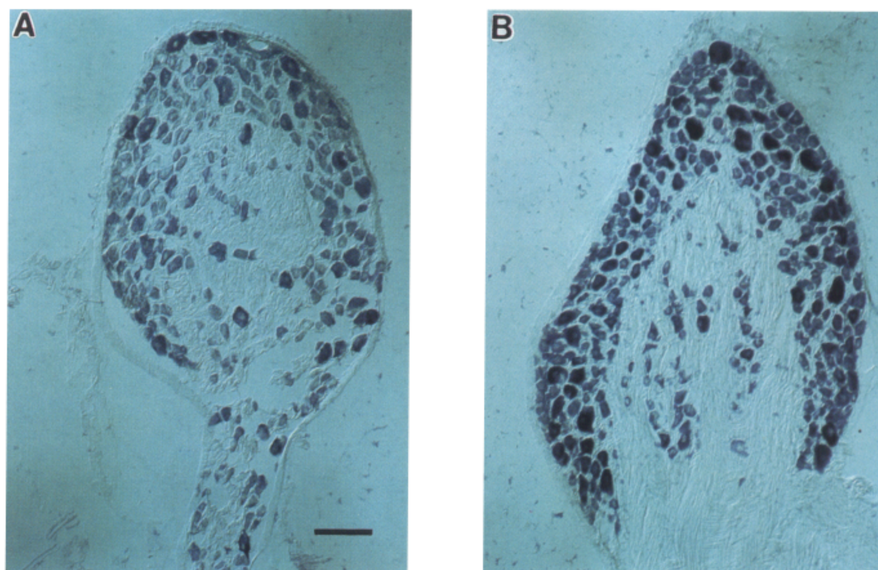

Figure 8. *In situ* hybridization analyses of *trkA* mRNA. DRG sections (20 μm thick), obtained from vehicle- and estrogen-treated OVX animals, were hybridized with a digoxigenin-labeled oligonucleotide probe for *trkA* mRNA. The extent of hybridization to the *trkA* probe was low in sensory neurons of vehicle-treated OVX animals (*A*), but increased dramatically in OVX animals exposed to estrogen for 52 hr (*B*). Photographed with Nomarski optics. Scale bar, 100 μm.

from controls (OVX) in other instances (data not shown). In general, though, it appears that the direction and time course of estrogen receptor mRNA regulation in DRG neurons are similar to that seen in the central neural targets of estrogen.

While the hormonal conditions during proestrus and estrogen replacement of OVX animals both downregulate estrogen receptor mRNA, they appear to have different effects on the two NGF receptor mRNAs present in DRG neurons. Both NGF receptors are present in DRG neurons: the $p75^{NGFR}$ protein, the first cloned NGF receptor (Chao et al., 1986; Johnson et al., 1986; Radeke et al., 1987), and *trkA* (Martin-Zanca et al., 1990). Compared to OVX animals, expression of *trkA* mRNA was enhanced comparably in both the proestrus animal as well as the estrogen-replaced OVX animals. *trkA* mRNA expression was significantly higher than controls 4 hr after estrogen treatment and remained upregulated 52 hr after estrogen treatment. There appeared to be no statistically significant increment in *trkA* mRNA expression between 4 and 52 hr of estrogen treatment, although at the present time we cannot determine whether estrogen acts by continuous stimulation of the *trkA* gene or by increasing the half-life of its transcript. While *trkA* mRNA levels remained equally high at both time points, estrogen titers at 52 hr postinjection were threefold lower than at 4 hr postinjection. Hence, unlike $p75^{NGFR}$ mRNA, which fell at 4 hr of estrogen treatment and returned to basal levels at 52 hr, *trkA* mRNA levels appear not to have fallen in response to decreasing availability of estrogen. However, since estrogen levels did not fall to basal (OVX) levels by 52 hr we can only speculate that even the low levels of estrogen present were capable of stimulating this gene. To test accurately whether *trkA* mRNA levels fall if estrogen is subsequently removed would be more feasible in a tissue culture system where exposure and deprivation of a ligand are more feasible.

While *trkA* mRNA was upregulated in both the proestrus and estrogen replacement condition, $p75^{NGFR}$ mRNA, on the other hand, which appeared enhanced during proestrus, was downregulated 50% following estrogen treatment, as compared to OVX controls. The most obvious explanation for the discrepancy in $p75^{NGFR}$ mRNA regulation between the two paradigms lies in the fact that, although estrogen titers are very high at proestrus, it is not the only gonadal hormone present at this time. In addition to estrogen, there are high levels of testosterone, prolactin, and follicle-stimulating hormone present during the afternoon of proestrus (Brown-Grant et al., 1970; Neill, 1972; Butcher et al., 1974), which may influence transcription of NGF receptors. It may prove to be the case that estrogen interactions with other hormones present at proestrus may result in markedly different outcomes from that of estrogen acting alone, as in the estrogen-replaced OVX animals. Thus, the changes in NGF receptor mRNA during proestrus must represent a sum total of several hormones exerting diverse control of the same gene, although the similarity on the direction and amount of *trkA* mRNA regulation in both paradigms (OVX/proestrus and estrogen-replaced OVX) suggests that *trkA* mRNA upregulation during proestrus may be due specifically to the high titers of estrogen present at this time.

Although estrogen appears to have differential effects on the two components of the NGF receptor, its actions appear to be consistent with those reported for other members of the steroid–thyroid hormone receptor superfamily of transcription factors. For example, replacing testosterone, a related gonadal hormone, downregulates $p75^{NGFR}$ mRNA in Sertoli cells of the adult testis, an androgen target (Persson et al., 1990). In the brain, long-term (16–30 d) exposure to a silastic implant of estrogen is reported to decrease $p75^{NGFR}$ mRNA in the hippocampus and septum (Gibbs and Pfaff, 1992). Retinoic acid, another member of this superfamily of DNA-binding proteins, has been shown to increase ^{125}I-NGF binding in Lan-1 neuroblastoma cells (Haskell et al., 1991) and in developing chick sympathetic neurons (Rodriguez-Tebar et al., 1991) and to increase *trkA* mRNA in neuroblastoma cells (D. R. Kaplan, personal communication). In fact, our own preliminary data from PC12 cells indicate that estrogen upregulates *trkA* mRNA and downregulates $p75^{NGFR}$ mRNA (Sohrabji, Greene, Miranda, and Toran-Allerand, unpublished observations), to a similar extent as in the DRG.

At the present time, we can only speculate on the biological and functional significance of differential regulation of NGF receptor mRNAs by estrogen, since the precise role of each receptor is not clearly known. While $p75^{NGFR}$ and *trkA* individually bind NGF with low affinity, together they demonstrate a

high-affinity binding characteristic (Hempstead et al., 1991). Some evidence suggests, however, that cellular requirements for the two NGF receptors may differ. In a mutant form (nnr) of PC12 cells, which does not respond to NGF with neurite elongation (Greene et al., 1986), $p75^{NGFR}$ is present at the usual levels but *trkA* mRNA is severely depleted (Loeb et al., 1991). Transfecting these mutants with an expression vector encoding *trkA* cDNA reinstated the capacity for NGF-induced neurite outgrowth (Loeb et al., 1991), suggesting that *trkA* is necessary for neurotrophin-mediated signal transduction. Hence, if *trkA* is the signal transducing arm of the NGF receptor, estrogen, by increasing *trkA* mRNA in sensory ganglia, may be in a position to alter neuronal responsivity to NGF, by altering the ratio of $p75^{NGFR}$ and *trkA*, and consequently, the formation of the hypothetical NGF receptor complex as recently suggested by Chao and colleagues (Battleman et al., 1993). In addition, it is interesting to note that preliminary evidence from transfected NIH-3T3 cells indicates that overexpression of $p75^{NGFR}$ inhibits autophosphorylation of *trkA* (D. R. Kaplan, personal communication). It may be that estrogen, by increasing *trkA* mRNA while decreasing $p75^{NGFR}$ mRNA, may modulate ligand-independent signal transduction by receptor tyrosine kinases. Although the present study focused on estrogen regulation of neurotrophin receptors that bind NGF, DRG neurons also express *trkB* and *trkC*, which bind other members of the neurotrophin family of growth factors. Localization of estrogen receptors in neurotrophin target neurons both in the DRG and CNS suggests that estrogen may be in a position to regulate mRNA expression of other tyrosine kinase receptors as well.

Estrogen replacement may downregulate $p75^{NGFR}$ mRNA in neurotrophin targets such as the DRGs and the basal forebrain (Gibbs and Pfaff, 1992) of the uninjured adult, but its actions on this gene may be different during periods of critical neurotrophin requirement such as development or following injury. For example, early neonatal exposure to estrogen appears to increase high-affinity NGF receptor binding, as determined by ^{125}I-NGF binding in DRG neurons (Wright et al., 1988), suggesting that estrogen may increase both *trkA* and $p75^{NGFR}$ transcriptionally or posttranscriptionally. Moreover in the adult brain, where estrogen downregulates $p75^{NGFR}$ mRNA in the intact basal forebrain, it appears not to augment the loss of $p75^{NGFR}$ mRNA, following fimbria-fornix lesions (Gibbs and Pfaff, 1992). Furthermore, estrogen treatment has been reported to have a beneficial effect on peripheral nerve regeneration (Bajusz, 1959), consistent with the hypothesis that estrogen, via interactions with growth factors known to ameliorate injury in the adult PNS, such as the neurotrophins, may have a protective role following neural injury.

The modulation of neurotrophin receptor mRNAs by gonadal hormones, particularly estrogen, in adult peripheral neural targets of NGF suggests a critical role for these steroids in mediating NGF-dependent regulation of neuronal survival, plasticity, and repair. Evidence from this laboratory indicates that estrogen receptor mRNA or protein colocalizes with *trkA* and *trkB* mRNA as well as $p75^{NGFR}$ mRNA or protein in neurotrophin targets of the developing forebrain (Toran-Allerand et al., 1992a; Miranda et al., 1993), suggesting the potential for estrogen regulation of NGF receptors in the CNS, as well as the potential for estrogen regulation of other members of the tyrosine kinase family of neurotrophin receptors. Moreover, since $p75^{NGFR}$ binds BDNF and NT-3 as well as NGF, gonadal hormones may also alter responsivity of target neurons to other members of the neurotrophin family. Steroid/neurotrophin interactions suggest a mechanism by which transcription factors such as the estrogen receptor, via actions on other transcription-regulating factors, may exert wide influences on the nervous system.

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