Expression of nerve growth factor receptor mRNA during early development of the chicken embryo: emphasis on cranial ganglia

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Summary

In situ hybridization with β-nerve growth factor receptor (NGF-R) oligonucleotide probes was used to study NGF-R mRNA expression in early chicken embryos. Sections through the region of the visceral arches showed high levels of NGF-R mRNA in mesenchyme of the visceral arches, neural tube and myotomes. Labeling was also seen over E3 primordium of the trigeminal ganglion (V) and in the placodal thickening of the petrosal (IX) and nodose (X) ganglionic primordia. In the E5 embryo, all cranial sensory ganglia (V, VII, VIII, IX, X) expressed NGF-R mRNA although at varying levels with higher levels in the ganglia of the Vth, IXth and Xth cranial nerves than in ganglia of the VIIth and the VIIIth nerves. Within ganglia of the Vth, IXth and Xth cranial nerves, levels of NGF-R mRNA were higher in regions containing placode-derived neurons, than in regions with neural-crest-derived neurons. The placode-derived nodose ganglion (X) expressed NGF-R mRNA at all stages of development. In the E15 embryo and later in development, two thirds of the large neuron-like cells expressed high levels of NGF-R mRNA. Our results show that expression of NGF-R mRNA, in peripheral neurons, is not restricted to cells of neural crest origin. We also show a transient expression of NGF-R mRNA early in development in a wide range of non-neuronal differentiating cells. The high level of NGF-R mRNA in early differentiating tissues suggest that the NGF-R plays a wider role during development than previously anticipated.

Key words: nerve growth factor receptor, in situ hybridization, chicken embryo, neural crest, placode, cranial ganglia.

Introduction

β-nerve growth factor (NGF) is a target-derived protein that is essential for development and maintenance of sympathetic and a subset of sensory peripheral neurons (see Levi-Montalcini and Angeletti, 1968; Thoenen and Barde, 1980; Levi-Montalcini, 1987). NGF has also been detected in the central nervous system (CNS) (Korsching et al. 1985; Shelton and Reichardt, 1986; Whittemore et al. 1986), where it supports cholinergic neurons in the basal forebrain (see Thoenen et al. 1987; Whittemore and Seiger, 1987; Ebendal, 1989). The neurotrophic effects of NGF on peripheral and central neurons are mediated by a membrane-linked cell surface receptor (NGF-R) (Banerjee et al. 1973; Herrup and Shooter, 1973; Hefti et al. 1986; Richardson et al. 1986; Taniuchi et al. 1986). The NGF-R can exist in two apparent states, the low- and high-affinity receptors, that have different kinetics of NGF binding (Sutter et al. 1979; Landreth and Shooter, 1980; Riopelle et al. 1980; Schechter and Bothwell, 1981). The high-affinity receptor mediates the biological activity of NGF and is therefore present on NGF responsive cells. However, several cell types, including Schwann cells have been found that carry only the low-affinity receptor (DiStefano and Johnson, 1988; Taniuchi et al. 1988).

Molecular cloning and analysis of complementary DNA (cDNA) clones for rat and human NGF-R (Johnson et al. 1986; Radeke et al. 1987) have shown that the receptor is a 396 amino-acid long protein with a single membrane spanning domain. The human NGF-R gene consists of six exons spanning 23 kilo basepairs (kb) on human chromosome 17 (Huebner et al. 1986; Rettig et al. 1986; Sehgal et al. 1988). The mRNA from this gene encodes the protein that forms part of both low- and high-affinity states of the NGF-R (Hempstead et al. 1989). More recently, both genomic and cDNA clones have been isolated for chicken NGF-R, which show considerable sequence homology with rat and human NGF-R (Ernfors et al. 1988; Large et al. 1989).

In agreement with the dependence of peripheral sympathetic and sensory neurons on NGF for develop-
ment and maintenance, these neurons produce NGF-R mRNA (Buck et al. 1987; Ernfors et al. 1988), exhibit specific binding of 125I-NGF (Rohrer and Barde, 1982; Raivich et al. 1985) and show NGF-R immunoreactivity (Yan and Johnson, 1987) throughout their embryonic and adult life. The cranial ganglia, including the nodose ganglion, have also been shown to bind 125I-NGF (Raivich et al. 1987), and to have NGF-R immunoreactivity on their surface (Yan and Johnson, 1987). Furthermore, the embryonic day 8 nodose ganglion in the chicken has been shown to express NGF-R mRNA (Ernfors et al. 1988). However, somewhat conflicting data have been reported concerning the responsiveness of placode-derived cranial sensory neurons to NGF stimulation. Although none of these ganglia respond to NGF in adult life, it appears as if, at least, the nodose ganglion shows a limited responsiveness to NGF early during development (Hedlund and Ebendal, 1980; Davies and Lindsay, 1985; Lindsay and Rohrer, 1985; Lindsay et al. 1985; Pearson et al. 1983).

In this study, we report on the regional and temporal expression of NGF-R mRNA in embryonic day 3 and 5 chick embryo, focusing on the cranial ganglia. In the nodose ganglion, expression of NGF-R mRNA was also studied during later stages of development. Our results show that NGF-R mRNA is expressed in a variety of cells during early embryogenesis including both neural-crest- and placode-derived cells. In non-neuronal tissues and in CNS, the levels of NGF-R mRNA decreased with differentiation, whereas, in some neurons derived from the neural crest and epibranchial placodes, high levels of NGF-R mRNA are maintained throughout development.

Materials and methods

Preparation and cryostat sectioning of chicken embryos

Fertilized White Leghorn eggs were incubated at 38°C for the desired time periods and stages were determined according to Hamburger and Hamilton (1951). Embryos younger than 12 days were frozen on dry ice whereas the upper thorax and neck regions were dissected and frozen from older embryos. 10 μm cryostat sections were collected on poly-L-lysine (60 μg ml⁻¹) pretreated glass slides. The sections were subsequently fixed in 4% paraformaldehyde for 30 min at 4°C, rinsed 2×1 min in PBS, dehydrated in a graded series of ethanol and in chloroform. The sections were finally air-dried and stored at -20°C before use for in situ hybridization.

In situ hybridization

Two oligonucleotides complementary to chicken NGF-R mRNA, corresponding to amino acids 47 to 60 (5'-CGTGCAGGGGC TTGACCGTT CTGTGCACATC CACAGTGTCC) and amino-acids 200 to 215 (5'-CGGCTCAGGA CGGCGTGCCA GCTGCCCATG ACGGGTGTGA CAATG)T, were synthesized on a DNA synthesizer (Applied Biosystem 381A). These oligonucleotides are referred to as probe 1 and 2, respectively. As a hybridization control, a 46-mer oligonucleotide complementary to probe 2 was also synthesized. It is referred to as the control probe. The oligonucleotides were labelled at their 3'-end with α[35S]dATP using terminaldeoxyribonucleotidyl transferase (Promega, WI) to a specific activity of 2×10⁶ cts min⁻¹ μg⁻¹ and purified on a Nensorb column (Dupont, Wilmington, DE) prior to use. Hybridization was performed at 42°C for 15 h in a humidified chamber with 150 μl of hybridization cocktail containing 1×10⁶ cts min⁻¹ of respective probe. The hybridization cocktail contained 50% formamide, 4×SSC (1×SSC is 0.15 M sodium chloride, 0.015 M sodium citrate pH 7.0), 10% dextrane sulphate (Pharmacia), 0.5 mg/ml yeast tRNA, 0.06 M dithiothreitol and 0.1 mg/ml 1-sonicated salmon sperm DNA. After hybridization, the slides were rinsed and washed 4×15 min at 55°C in 1×SSC, 2×1 min in cold RNase-free water, dehydrated in ethanol and left to air-dry. The slides were dipped in Kodak NTB-2 photographic emulsion diluted 1:1 in 0.6 M ammonium acetate and exposed for 15 days at -20°C. The slides were then developed and fixed, and the sections lightly counter stained with cresyl-violet, mounted and examined in a photomicroscope.

Results

Expression of NGF-R mRNA in E3 chicken embryo

Sections were prepared from E3 chicken embryos (Hamburger and Hamilton stage 18) in the rhombencephalic region through the hyoid arch (second visceral arch). Hybridization with the specific NGF-R probes to sections from the region between the metencephalon and the anterior intestinal porta showed very intense labelling over the lateral and ventrolateral mesencephyme of the visceral arches (Fig. 1B). Less intense labelling was seen over the ventromedial parts of all arches (Fig. 1A,B,C). The mesencephyme of the ventromedial parts of the first visceral arch and the hyoid arch was less intensely labelled than the corresponding region in the more caudal visceral arches (Fig. 1B,D). Weak labelling was also seen over the sclerotomic mesenchyme.

Intense labelling was seen over the neural tube in the studied region (myelencephalon and anterior spinal cord) without any variation along the rostrocaudal axis. Labelling in the dorsal parts of the neural tube was less intense than over the ventral parts and no labelling was seen over the thin roof of the myelencephalon. Labelling in the ventromedial parts of the neural tube was weaker than labelling over the lateral mesenchyme (Fig. 1). The labelling was equally distributed over the layers of the neural tube (Figs 1, 2B).

Specific labelling was seen over a limited area of the ectodermal epithelium of the most lateral and central part of the otic vesicle (Fig. 2A). No labelling was detected over the epidermal ectoderm of the head and trunk except for the epidermal thickening of the primordia of the petrosal (IX) (Fig. 3) and nodose (X) ganglia. No labelling was detected over endodermal epithelium of the pharynx, pharyngeal pouches and gut. The same was true for endothelia of blood vessels and E3 heart endomyocardium (Fig. 1B,C).

Expression of NGF-R mRNA in E5 chicken embryo

Sections were cut from E5 chicken embryo (Hamburger and Hamilton stage 26), parallel to the third aortic arch.
Fig. 1. Expression of NGF-R mRNA in branchial arches in the E3 chicken embryo. Sections through (A) the first visceral arch and the rostral telencephalon, (B) the otic vesicles and the second visceral arch and (C) the third and fourth visceral arches of the E3 chicken embryo shown by dark-field microscopy after hybridization to the NGF-R probe 2. (D) A section through the third visceral arch hybridized to the control probe (dark-field micrograph). (E) Schematic representation of section B through the otic vesicles and the second visceral arch. Cross-hatched area delineates the wall of the neural tube. (F) Schematic representation of sections A, B and C. Dorsal aorta (a), heart (h), neural tube (nt), otic vesicle (o), pharynx (ph), telencephalon (t). Bar 250 μm.
in the region between the upper oral cavity and the lower parts of the heart. Very intense labelling was seen over the ventrolateral mesenchyme of the hyoid arch where the arch expands caudally over the third visceral arch (Fig. 4A, B). Intense labelling was also seen over the differentiating myotome. Considerably weaker labelling was seen over the dermato- myotome (Fig. 4C). No labelling was seen over mesenchyme in E7 embryo or at later stages of development (data not shown). Labelling was seen over retina, eye muscle anlagen, neural tube, peripheral ganglia, nerves and the otic vesicle in E5 embryo. Labelling over the retina was weak (Fig. 5C, D), mainly localized to the ganglion cell layer but a faint signal was also seen throughout the thickness of the retina (Fig. 5C, D). No specific signal could be detected over the pigment epithelium (Fig. 5D).

The level of NGF-R mRNA in E5 medulla oblongata was higher than that in the E3 rhombencephalic neural tube (compare Figs 2B and 5B). In the lower metencephalon at the level of the trigeminal ganglion, weak labelling was seen over the ventricular zone consisting of proliferating neuroepithelial cells (Fig. 5B). The inner mantle layer was intensely labelled, surrounded by the less intensely labelled outer mantle. At this level of the brainstem, the alar plate was more intensely labelled than the basal plate corresponding to areas with developing motornuclei (Heaton and Moody, 1980). No NGF-R mRNA was detected in fiber bundles in the marginal zone of brain stem, but meninges surrounding the brain stem were weakly labelled (Figs 4C, 6B, D). This labelling increased more caudally in the studied area.

The same labelling pattern over the neural tube was seen at the level of the jugular ganglion (Xth proximal ganglion) (Fig. 6D) and the trigeminal ganglion (Vth
Fig. 4. Detection of NGF-R mRNA in E5 mesectoderm and mesoderm. (A) Section through the lower part of the hyoid arch (second visceral arch) hybridized to the NGF-R probe 2. (B) Magnification of the area indicated by the arrow in panel A showing labelled mesenchyme and unlabelled epithelium. (C) Transverse section through the spinal cord and myotomes in the upper thoracic region. Third aortic arch (a), hyoid arch (ha), myotome (m), pharynx (ph), spinal cord (sc). Bars in A, C, 250 μm and in B, 25 μm.

cranial nerve) (Fig. 5B). An increased level of NGF-R mRNA was seen in the most ventromedial part of the inner zone (Figs 5A, B, 6B, D). Fiber bundles at the outer ventromedial aspect of the brain stem did not show any labelling (Fig. 5A, B).

Specific labelling was also seen in the E5 developing otic vesicle forming the inner ear labyrinth (Fig. 6B, C).

The labelling was restricted to specific areas of the inner epithelium corresponding to the primordium of the ampulla and the superior semicircular canal as well as to the primordium of the lateral semicircular canal. In general, the most ventrolateral and superior parts of the developing otic vesicle showed labelling with the NGF-R probe. No labelling was seen over the epithelium of the otic vesicle close to the acoustico-facial ganglion complex (Fig. 6A, B). Similarly, labelling was not detected over the endodermal epithelium of pharynx or gut, endothelium of blood vessel or over epidermis. No labelling was detected over the primordium of thymus which is derived from the epithelium of the third and fourth visceral pouches, or the bilobed thyroid.

**NGF-R mRNA expression in cranial sensory ganglia**

E3 chicken embryos (Hamburger and Hamilton stage 17-18) were sectioned transversely at the third visceral arch region. The primordium of the trigeminal (V) ganglion located between the neural tube and the epidermis (Fig. 2B) was clearly labelled. However, labelling over the ganglion was diffuse and individually labelled cells could not be identified. Weak labelling was seen over the primordia of the distal ganoglia of the IXth (Fig. 3) and Xth cranial nerves. Labelling was also seen over the ectodermal thickenings of the epibranchial placodes, whereas no labelling was seen over fibers projecting towards the rhombencephalic neural tube (Fig. 3A).

All E5 cranial sensory ganglia expressed NGF-R mRNA in varying amounts, as shown in Table 1. The distal parts of the trigeminal (V) ganglion, corresponding to the ophthalmic and maxillo-mandibular lobes, were intensely labelled (Fig. 5A, B). The labelling over the proximal portion of the ganglion, where the two lobes are fused together, was not as intense as over the distal parts.

Faint NGF-R mRNA expression was seen in the acoustico-facial (VII, VIII) ganglionic complex (Fig. 6A, B). Weak, but somewhat higher, labelling was

### Table 1. Expression of NGF-R mRNA in E5 sensory cranial ganglia

<table>
<thead>
<tr>
<th>Ganglion</th>
<th>Relative labelling1</th>
</tr>
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<tbody>
<tr>
<td>Trigeminal (V)</td>
<td></td>
</tr>
<tr>
<td>d. Maxillo-mandibular lobe²</td>
<td>+++</td>
</tr>
<tr>
<td>d. Ophthalmic lobe²</td>
<td>+++</td>
</tr>
<tr>
<td>p. Trigeminal²</td>
<td>++</td>
</tr>
<tr>
<td>Acoustico-facial (VIII, VII)</td>
<td></td>
</tr>
<tr>
<td>Acoustic part</td>
<td>+</td>
</tr>
<tr>
<td>Vestibular part</td>
<td>(+)</td>
</tr>
<tr>
<td>Geniculate (VII)</td>
<td>+</td>
</tr>
<tr>
<td>Superior (p. IX)</td>
<td>+</td>
</tr>
<tr>
<td>Petrosal (d. IX)</td>
<td>++</td>
</tr>
<tr>
<td>Jugular (p. X)</td>
<td>+</td>
</tr>
<tr>
<td>Nodose (d. X)</td>
<td>+++</td>
</tr>
</tbody>
</table>

1 ++ + Intense labelling, ++ less intense labelling, + weak labelling, (+) faint labelling.  
2 The border between the proximally and distally labelled parts of the ganglion was not clear.  
3 d. distal, p. proximal.
seen over the geniculate (VII) ganglion and over the caudal part of the acoustic ganglion, close to the otic vesicle.

Weak labelling was also seen over the superior (IX) ganglion and the jugular (X) ganglion (Fig. 6D). Intense labelling was seen over the distal ganglia of the IXth and Xth cranial nerves. The nodose (X) ganglion was more intensely labeled than the petrosal (IX) ganglion.

The mandibular nerve was weakly labelled by the NGF-R probe (Fig. 6B). Labelling was seen along the nerve out to the fine processes near the epidermis over the hyoid arch. In contrast to the mandibular nerve, the vagus and the glossopharyngeal nerves were not labelled by the NGF-R probe.

**Developmental expression of NGF-R mRNA in the nodose (X) ganglion**

Sections through the nodose ganglion from E5, E7, E9, E12, E15, E17, E19 and P3 were analyzed. In the E5 nodose ganglion, intense, but diffuse, labelling was seen (Fig. 7A) and individually labelled cells could not be identified. An intense and evenly distributed labelling was seen over the E7 ganglion. In the E9 nodose ganglion, larger cells could be distinguished and in the E12 ganglion (Fig. 7B) labelling was concentrated over the larger cells, corresponding to differentiating neurons. In the E17 ganglion, labelling with varying intensities was seen over the large neuron-like cells (Fig. 7C,D). Non-labelled large cells were first observed at E15 and comprised approximately one third of all large cells. The proportion of non-labelled cells was similar in E17 as in P3 ganglia. Specific labelling was not detected over small non-neuronal cells in the nodose ganglion from stage E15 and at later times of development (Fig. 7D,F,G).

**Specificity of the in situ hybridization analysis**

All results described above were obtained by hybridizing every second consecutive section to the specific NGF-R probe 2. The remaining sections were hybridized to either the specific NGF-R probe 1 or to the control probe. The labelling pattern obtained with probe 1 was identical to the pattern seen with probe 2. No hybridization was seen on any of the sections using the control probe (see examples in Figs 1D, 5D, 7E).

**Discussion**

The present in situ hybridization analysis in the chicken embryo detected NGF-R mRNA in classical NGF target cells as well as in many tissues not known to be responsive to NGF. Two different non-overlapping probes were used to detect NGF-R mRNA expressing cells and one probe was used to assess the specificity of labelling. The specific NGF-R probe 2 hybridizes to the region encoding the membrane spanning domain in chicken NGF-R mRNA. This domain is highly conserved in rat, human and chicken, with a 95% homology between human and chick amino-acid sequences and 81% homology in DNA sequences (Ernfors et al. 1988; Large et al. 1989). The specific NGF-R probe 1 is from the region encoding the first extracellular cysteine-rich domain, which is less conserved during evolution. Both probes are therefore specific for the NGF-R, but we can not exclude the possibility of cross-hybridization
Fig. 6. Expression of NGF-R mRNA in E5 cranial ganglia and the otic vesicle. Section through the acoustico-facial ganglion complex and rostral part of the developing otic vesicle shown by (A) bright-field illumination and (B) dark-field illumination after hybridization to the NGF-R probe 2. (C) Magnification of a section through the otic vesicle epithelium. (D) Section through the jugular ganglion. The dashed line indicates the outer border of the ganglion. Acoustico-facial ganglion complex (a-f), geniculate ganglion (g), jugular ganglion (j), otic vesicle (ov), the mandibular nerve (n), neural tube (nt). Bars in A, B, D 250 μm and in C, 25 μm.
Fig. 7. Developmental expression of NGF-R mRNA in the nodose ganglion. Bright-field illuminations of sections from the mid part of the nodose ganglia from chicken embryos after hybridization to the NGF-R probe 2. Nodose ganglion from (A) E5, (B) E12 and (C) E17 chicken embryo. (D) Higher magnification of the boxed area in panel C, showing labelled E17 nodose neurons. (E) Control probe used on section through E17 nodose ganglion. (F) P3 nodose ganglion at low magnification. (G) Higher magnification of P3 nodose ganglion cells. Filled arrow: intensely labelled neuron-like cell. Hollow arrow: non-labelled neuron-like cell. Bars in F, 150 μm, in A, B, C 50 μm and in D, E, G 25 μm.
to a putative very closely related, not yet identified, receptor gene.

The two NGF-R specific oligonucleotide probes showed identical labelling patterns on adjacent sections, strongly supporting a correct NGF-R mRNA specificity. The specificity of the NGF-R probe 2 has also been tested by Northern blot hybridization where it only recognized a 4.5 kb chicken NGF-R mRNA (Ernfors et al. 1988). The control probe did not show any specific or unspecific labelling.

NGF-R mRNA has previously been detected by Northern blot analysis in several tissues of the chicken embryo (Ernfors et al. 1988; Escandon and Chao, 1989; Large et al. 1989). The widespread occurrence of NGF-R mRNA in the early chicken embryo prompted us to study the cellular distribution of NGF-R mRNA, focusing on regions including developing neural crest, placodes and their derivatives.

Our results show NGF-R mRNA in both E3 and E5 mesenchymal and neuro-ectodermal cell types undergoing differentiation. A previous study has shown binding of 125I-NGF (Raivich et al. 1985) to mesenchymal cell types of myotomes and muscle anlagen and the specific NGF-R probes also revealed labelling over these cells both in the E3 and E5 embryo. In addition, we detected high levels of NGF-R mRNA in the pharyngeal region, in ventrolateral mesenchyme of the visceral arches, whereas no labelling was found more caudally over the somatopleura and splanchnopleura. The mesenchymal cells of the visceral arches are mainly of neural crest origin (LeLièvre and Le Douarin, 1975; Noden, 1975; Le Douarin, 1980; Ayer-LeLièvre and Le Douarin, 1982) and the cells expressing high levels of NGF-R mRNA in this region are therefore mainly undifferentiated mesectodermal cells that have stopped migrating but are proliferating. It has been shown that neural crest cells bind 125I-NGF when cultured in vitro, implying that expression of NGF-R coincides with the time when these cells acquire their phenotypic characteristics (End et al. 1983; Bernd, 1985; Greiner et al. 1986). It is not known if the onset of NGF-R mRNA expression is a result of the first steps of differentiation or if it is a necessary event for the differentiation to occur. However, since NGF-R mRNA was detected in postmigration undifferentiated neural crest cells, it appears that the onset of NGF-R mRNA expression occurs before these cells acquire their phenotypic characteristics. Our results also show that the level of NGF-R mRNA in mesenchymal cells of the visceral arches is dramatically decreased after embryonic day 5 when differentiation of myoblasts and prechondrocytes from visceral arch mesenchyme starts, suggesting that the NGF-R plays a role in the early development of this tissue. This property may either be mediated by binding of NGF to the receptor or, alternatively, the receptor may promote cellular interactions during differentiation by a ligand-independent mechanism.

A transient expression of NGF-R mRNA was also observed in the developing rhombencephalon and cervical spinal cord with no or very low levels of NGF-R mRNA later in development. In the embryonic rat spinal cord, NGF-R mRNA has been detected in motoneurons but expression of NGF-R mRNA in adult rat motoneurons is reduced below the detection limit of in situ hybridization (Ernfors et al. 1989). In the adult rat, NGF has been shown to bind to a few cells in lower brainstem and medulla oblongata (Richardson et al. 1986). However, the role of NGF-R in these neurons is unknown.

The large developmental changes in the levels of NGF-R mRNA in the chicken embryo, as well as in rat (Buck et al. 1987; Yan and Johnson, 1987; Ernfors et al. 1988, 1989), indicate that expression of the NGF-R gene is developmentally regulated. DNA sequences upstream of the transcriptional start site in the human NGF-R gene lack a TATA element found in the promoter region of most eukaryotic genes (Breathnach and Chambon, 1981). Instead, the NGF-R promoter region show several features characteristic of constitutively expressed genes (Sehgal et al. 1988). Chronic infusion of NGF into the adult rat forebrain results in an increased number of NGF-R mRNA expressing cells in the basal forebrain suggesting that NGF can upregulate expression of its own receptor (Higgins et al. 1989). Furthermore, recent data have shown that expression of NGF-R mRNA in rat Sertoli cells is downregulated by testosterone implying that steroid hormones may also regulate NGF-R mRNA expression in other tissues (Persson et al. 1990). Interestingly, the two enzymes 5-a-reductase and aromatase, which metabolize testosterone to the more potent metabolites dihydrotestosterone and estradiol, respectively, are both widely distributed in fetal tissues (MacLusky and Naftolin, 1981; McEwen, 1983). One possible target for fetal testosterone could be the NGF-R gene, which may be downregulated in response to the steroid, thereby influencing development of both neuronal and non-neuronal tissues.

Our results show NGF-R mRNA expression in placodes of developing cranial sensory ganglia and in the placode-derived otic vesicle. In contrast to the epibranchial placodes, NGF-R mRNA expression in the otic vesicle is not located in the epithelium close to the primordium of the ganglion. In E3 embryo (stage 18) NGF-R mRNA is expressed in the lateral part of the vesicle (Figs 1B, 2A) and cells giving rise to the acoustico-facial ganglionic complex bud from the ventromedial aspect of the otic vesicle (D’Amico-Martel and Noden, 1983). It is therefore unlikely that the placode-derived cells in the otic vesicle expressing NGF-R mRNA form part of the primordium of the acoustico-facial ganglion. In the case of the developing distal ganglia of the IXth and Xth cranial nerves the placode-derived cells form part of the ganglia. The observation that NGF-R is expressed in the epithelium of the otic vesicle agrees with Raivich et al. (1987) and Represa and Bernd (1989). The localization is different from that described by Raivich who finds 125I-NGF binding on the dorsal and superior part of the E4 chick embryo otic vesicle, and Bernd who finds binding in the ventromedial aspect of the otic vesicle from 72 h old quail embryos. The different localization of the NGF-R
expressing cells can be due to differences in stage and species. In spite of somewhat divergent results, it is clear that NGF-R is expressed in restricted parts of the epithelium of the otic vesicle and the NGF-R on these cells probably influences development of the inner ear epithelium (Represa and Bernd, 1989) rather than being involved in development of the acoustico-facial ganglionic complex.

All cranial sensory ganglia from early stages of development expressed NGF-R mRNA although in varying amounts. In contrast to NGF-R mRNA expression in the mesenchyme and neural tube, the levels of NGF-R mRNA remain high in cranial ganglia also at later stages of development. In the E5 chick embryo, all sensory cranial ganglia show a weak response to NGF, whereas at later times during development mostly neural-crest-derived neurons respond to NGF by fiber outgrowth (Davies and Lindsay, 1985). Not all neural-crest-derived neurons respond to NGF even though they express NGF-R as shown by neurons from the trigeminal mesencephalic nucleus grown in culture (Davies et al. 1987). This is the situation in the trigeminal ganglion (V), in which case NGF sensitivity appears to be restricted to the proximal part of the ganglion where neural-crest-derived neurons are located (Ebendal and Hedlund, 1974, 1975; Davies and Lumsden, 1983). However, in the trigeminal ganglion higher levels of NGF-R mRNA expression were seen in the distal parts of the ganglion where placode-derived neurons are located. A similar situation was seen in ganglia of the glossopharyngeal (IX) and vagus (X) nerves, where neurons of the petrosal (IX) and nodose (X) ganglia are placode-derived while neurons of the superior (IX) and jugular (X) ganglia are derived from the neural crest. Several reports have shown that developing neurons in the nodose ganglion do not respond to NGF (Davies and Lindsay, 1985; Davies et al. 1986; Lindsay and Rohrer, 1985), except for a brief time-period early in development (Hedlund and Ebendal, 1980; Ebendal and Persson, 1988). Injection of NGF in the fertilized chicken egg did not increase the number of surviving nodose neurons in the developing embryos (Dimberg et al. 1987) and immunodepletion of NGF during intrauterine development in guinea pig had no effect on the number of developing nodose neurons (Pearson et al. 1983). This strongly suggests that neurons from the nodose ganglion are less dependent on NGF than sympathetic and sensory neurons. However, the higher levels of NGF-R mRNA found in the placodal neurons of the nodose and petrosal ganglia, compared to the jugular and superior ganglia, suggest that the NGF-R is functionally relevant for these neurons. This is further supported by the fact that during maturation of the nodose ganglion, a segregation in the neuronal population takes place where approximately two-thirds of the neurons contain high levels of NGF-R mRNA whereas the remaining neurons in the ganglia do not contain any detectable NGF-R mRNA.

A possible explanation for the high levels of NGF-R mRNA in a subpopulation of nodose neurons could be that the NGF-R mediates a trophic response in these cells by interacting with a ligand different from, but yet structurally related to, NGF. The nodose ganglion responds to brain-derived neurotrophic factor (BDNF) (Davies and Lindsay, 1985; Davies et al. 1986) and, interestingly, the BDNF protein sequence, deduced from a recently isolated cDNA clone (Leibrock et al. 1989), shows high sequence homology to the NGF protein. Moreover, binding of BDNF to its receptor show many characteristics in common with binding of NGF to its receptor, including two receptor affinity classes \(K_{d(\text{low})} 1.3 \times 10^{-7} \text{M}, K_{d(\text{high})} 1.7 \times 10^{-11} \text{M}\) (Rodriguez-Tebar and Barde, 1988). Considering these intriguing data one can speculate that BDNF may also bind to the NGF-R present in a 'BDNF high-affinity state' and thereby mediating a response for developing nodose ganglia. Another plausible possibility is that, although NGF is not essential for survival of neurons in the nodose ganglion, an interaction of the factor with its receptor may for instance stimulate neurotransmitter metabolism or receptor synthesis in these neurons.

The developmentally regulated expression of NGF-R mRNA in a wide range of different tissues in the early chicken embryo suggests that the NGF-R plays a role in the development of a variety of different tissues both of neuronal and non-neuronal origins. The mechanisms by which the NGF-R functions in such a wide range of different tissues remain to be elucidated.

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