EVIDENCE FOR A ROLE OF NGF IN THE VISUAL SYSTEM

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INTRODUCTION

It is generally accepted that the development, maintenance and survival of specific neuronal populations, both in the peripheral (PNS) and central nervous system (CNS), is dependent upon the supply of diffusible trophic molecules, produced in limiting amounts by neurons and/or glia in their target fields (for review, see Thoenen et al., 1987). The prototype of neuronotrophic factors, Nerve Growth Factor (NGF) is essential for neural crest-derived sensory and peripheral sympathetic neurons (Levi-Montalcini and Angeletti, 1968) and for cholinergic neurons of forebrain nuclei in the CNS (Hefti, 1986; Vantini et al., 1989). In all these NGF-responsive peripheral and central neurons, NGF binds to specific cell surface receptors (NGFRs) expressed both on cell bodies and axonal terminals in the innervated target area (Greene and Shooter, 1980). The immunocytochemical mapping of the NGFR with the 192-IgG monoclonal antibody (Chandler et al., 1984) has demonstrated that the receptor is expressed by many different neuronal population in the CNS (Yan and Johnson, 1989; Pioro and Cuello, 1990), suggesting that NGF, or an NGF-like molecule have a trophic role for many other cell types beyond the cholinergic ones. The first evidence that NGF may also be active in the visual system was obtained in the early eighties when it was demonstrated that NGF, when intraocularly supplied to axotomized retinal ganglion cells (RGCs) in the goldfish, enhanced the process of axonal regeneration in the transected optic nerve (ON). More recent studies (Yan et al., 1989; Pioro and Cuello, 1990) have demonstrated that the NGFR is expressed in many nuclei of the visual system receiving a retinal input both in developing and adult rats.

In addition the messenger RNA encoding for NGFR has been reported to be present in the retina of avian (Large et al., 1989) and of many different mammalian species, including primates (Schatteman et al., 1988). It has been demonstrated that NGF, when intraocularly supplied to axotomized RGCs in the goldfish, enhances processes of axonal regeneration in the transected optic nerve (ON) (Turner et al., 1980; Yip and Johnson, 1982). These were the first evidence suggesting that NGF may also be active in the visual system.

Here we will review our results obtained over the last few years on the expression and distribution of NGFR in the rat visual system. We
will also briefly describe the effects produced by exogenously supply-
ing NGF to axotomized rat RGCs and to monocularly deprived kittens. All
together, these results allow us to hypothesize that NGF itself, or an
NGF-like molecule, plays an important role in the mammalian visual
system.

MATERIALS AND METHODS

Optic nerve (ON) section. Adult Long Evans rats had their right ON
intracranially transected. Animals received intraocular injections of
either NGF or cytochrome c (cyt c) (3 ug in 3 ul per injection on
either day). Seven weeks after ON section, the ONs were serially cut
and prepared for electron microscopy (for details, see Carmignoto et
al., 1989).

Monocular deprivation (MD). Under halothane anesthesia, kittens
were monocularly deprived by lid suture of the right eye at the 30th
day of age. At the same time a cannula connected to a 2002 Alzet mini-
pump filled with 0.5 ug/ul of either NGF or cyt c was inserted into
the anterior ventricle. Kittens were divided in control untreated
(n=2), cyt c (n=3) and NGF (n=3) treated groups.

After two weeks of MD, extracellular action potentials were re-
corded from single units of area 17 with tungsten microelectrodes
(Digitimer), conventionally filtered, amplified and audiomonitored.
Each cell recorded was assigned to one group of the seven point scale
of Hubel and Wiesel (1977). The degree of shift in ocular dominance
after monocular deprivation was expressed by the Binocularity (B),
which is defined as the number of cells in groups 2, 3, 4, 5, and 6
divided by the number of all visually responsive cells, and the open
eye dominance (OED) (Paradiso et al., 1983) in the hemisphere contra-
lateral to the open eye, as follows:

\[
OED = \left( \frac{n^o \text{ cells gr 1}}{\text{total } n^o \text{ cells}} \right) + \frac{2}{3} \left( \frac{n^o \text{ cells gr 2}}{\text{total } n^o \text{ cells}} \right) + \frac{1}{3} \left( \frac{n^o \text{ cells gr 3}}{\text{total } n^o \text{ cells}} \right)
\]

Symmetrical penetrations were made in both hemispheres.

RESULTS

NGFR in the visual system. We have recently demonstrated that in
the rat retina the messenger RNA encoding for NGFR is expressed through-
out development and in adulthood (Carmignoto et al., 1991). In addition
we have localized immunocytochemically localized NGFR by using the
192-IgG monoclonal antibody. As shown in Fig. 1A, NGFR immunoreactivity
in the adult rat retina is associated with Muller cell bodies located
in the inner nuclear layer (INL) and with their end feet processes in
the ganglion cell layer (GCL). Immunopositive cell bodies located in
the ganglion cell layer are also present. On the basis of soma diameter
and dendritic arborization at least some of these cells can be classi-
fied as RGCs. Fig. 1B shows an example of a cell with a soma diameter
of approximately 14 um. Fig. 1C shows an example of a cell with a soma
diameter of approximately 21 um and the typical arborization of a type
1 RGC.

At least some RGCs were also capable of anterogradely and retro-
gradely transporting the NGFR along optic nerve fibers (Carmignoto et
al., 1991). Our results are consistent with other studies in both
developing and adult rats where the NGFR-like immunoreactivity was
demonstrated to be mainly associated to fibers and terminals in all the
visually related nuclei (Yan and Johnson, 1989; Pioro and Cuello,
1990), i.e., the Superior Colliculus, dorsal and ventral part of the
Lateral Geniculate Nucleus, Suprachiasmatic nucleus, and nuclei of the