The Response of Chick Sensory Neurons to Brain-Derived Neurotrophic Factor

Alun M. Davies, H. Thoenen, and Yves-Alain Barde

Department of Anatomy, St. George's Hospital Medical School, London, and Department of Neurochemistry, Max-Planck-Institute for Psychiatry, Munich, West Germany

To determine the spectrum of activity of brain-derived neurotrophic factor (BDNF) among first-order sensory neurons, explants of the nine distinct populations of sensory neurons from embryonic chicks of 3-14 d incubation (E3-E14) were grown in collagen gels with and without BDNF in the culture medium. The explants responded to BDNF with profuse neurite outgrowth and comprised those in which neurons are derived from neural crest (the dorsomedial part of the trigeminal ganglion, rostromedial part of the trigeminal mesencephalic nucleus, jugular ganglion, and lumbosacral dorsal root ganglia) and from epibranchial placodes (the ventrolateral part of the trigeminal ganglion and the geniculate, petrosal, and nodose ganglia). This response was first clearly observed in the ventrolateral trigeminal and nodose ganglia as early as E4, but did not appear until later in other explants. The dorsomedial trigeminal, jugular, and geniculate ganglia were the latest to develop a response, which was not apparent until E8. In all explants the response was maximal between E10 and E12, and there was a decline in the magnitude of the response from E12 to E14. Although explants of the vestibular ganglion failed to respond to BDNF, the survival and growth of vestibular neurons in dissociated neuron-enriched cultures were promoted by BDNF. To investigate whether all first-order sensory neurons respond to BDNF or whether BDNF-responsive neurons comprise a distinct subset, we studied the influence of BDNF and NGF on the survival and growth of the placode-derived and the neural crest-derived neurons of the trigeminal ganglion in dissociated neuron-enriched culture. Whereas the great majority of ventrolateral trigeminal neurons (placode-derived) survived and grew in response to BDNF, only about 7% of dorsomedial trigeminal neurons (neural crest-derived) survived with BDNF. These findings demonstrate that although BDNF-responsive neurons are present in all populations of first-order sensory neurons, the proportion of responding neurons in these populations varies.

Sensory neurons connect specific types of sensory receptors in the periphery with particular groups of second-order neurons in the CNS. During the establishment of these connections, from 30 to 70% of the neurons originally present die (Oppenheim, 1981), and this loss is believed to match the number of neurons to the requirements of their target fields and to eliminate inappropriately connected neurons (Cowan et al., 1984). Considerable evidence, largely from work on NGF, suggests that neuronal survival during this critical period of development depends on the supply of a trophic factor conveyed from the target field

to the perikaryon by axonal transport (Berg, 1982; Thoenen and Barde, 1980).

The presence of NGF in developing cutaneous fields (A. M. Davies, S. Korsching, and H. Thoenen, unpublished observations) and in the peripheral, but not the central, branches of dorsal root ganglia (Korsching and Thoenen, 1985), together with the demonstration of the retrograde transport of endogenous NGF from the periphery (Korsching and Thoenen, 1983), suggests that NGF provides trophic support to developing sensory neurons via their peripheral processes. NGF does not, however, support the survival and growth of all types of sensory neurons: For example, the placode-derived neurons of the ventrolateral trigeminal (Davies and Lumsden, 1983; Ebendal and Hedlund, 1975), the nodose (Lindsay et al., 1985), geniculate, vestibular, and petrosal ganglia (Davies and Lindsay, 1985), and the neural crest-derived neurons of the trigeminal mesencephalic nucleus (A. M. Davies, A. G. S. Lumsden, H. Thoenen, and H. Rohrer, unpublished observations) do not respond. Although these sensory neurons are unresponsive to NGF, there is evidence that they are supported by other neurotrophic factors present in their peripheral target fields. The enteroceptive neurons of the nodose ganglion are supported by a factor present in liver (Lindsay and Tarbit, 1979), and the proprioceptive neurons of the trigeminal mesencephalic nucleus are supported by a factor present in skeletal muscle (Davies, in press). Furthermore, these as yet unpurified factors have a negligible neurite-promoting effect on explants of the NGF-responsive cutaneous sensory neurons of the trigeminal ganglion (Davies, in press; Davies and Lindsay, 1984). Taken together, the above studies suggest that each kind of sensory neuron is supported by one of a number of neurotrophic factors present in the periphery.

Recent experiments (Yip and Johnson, 1984) showing that central rhizotomy of dorsal root ganglia (DRG) in newborn rats results in the death of a proportion of the neurons suggest that sensory neurons also require trophic support from the CNS. Little is known, however, of the molecular basis of such support. Since sensory neurons project to a variety of different kinds of second-order neurons in the CNS, it is possible that, as in the periphery, a number of different neurotrophic factors are involved. One way to begin to investigate this possibility would be to determine the spectrum of activity of a CNS-derived neurotrophic factor for sensory neurons. Brain-derived neurotrophic factor (BDNF), which has been purified from pig brain (Barde et al., 1982), appears to be such a factor. It has been shown to support the survival and growth of nodose and dorsal root ganglion neurons in culture but is inactive on sympathetic and parasympathetic neurons (Lindsay et al., 1985).

Materials and Methods

Dissection

Electrolytically sharpened tungsten needles were used to dissect lumbosacral DRG and the cranial sensory ganglia (Davies and Lindsay,

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Correspondence should be addressed to Alun M. Davies, Department of Anatomy, St. George's Hospital Medical School, Cranmer Terrace, London SW17 0RE, England.

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Figure 1. Bar charts showing the magnitude of neurite outgrowth from ganglion explants of E3-E14 chick embryos. *Bar height*, Median of neurite outgrowth scores after 24 hr incubation; upper and lower limits of the vertical line transecting each bar, the positive and negative interquartile deviations, respectively. Three bars are drawn for each kind of explant: the first (*blank bar*) summarizes neurite outgrowth in control cultures; the second and third bars (*hatched*) summarize neurite outgrowth from explants grown in the presence of BDNF at 25 and 100 ng/ml, respectively. *TG*, Whole trigeminal ganglion at E3 only; *DM-TG*, dorsomedial pole of the maxillomandibular pole of the trigeminal ganglion; *VL-TG*, ventrolateral pole of the maxillomandibular lobe of the trigeminal ganglion; *MES*, rostromedian part of the trigeminal mesencephalic nucleus; *GEN*, geniculate ganglion; *VEST*, vestibular component of the vestibuloacoustic ganglion; *PET*, petrosal ganglion; *JUG*, jugular ganglion; *NOD*, nodose ganglion; *DRG*, dorsal root ganglion.

Figure 2. Graph summarizing the age-related trends in BDNF-promoted neurite outgrowth from neural crest-derived explants (solid symbols) and placode-derived explants (open symbols). The median of the neurite outgrowth scores after 24 hr in the presence of BDNF (100 ng/ml) is plotted. Solid triangles, Dorsomedial part of the trigeminal ganglion; solid diamonds, rostromedial part of the trigeminal mesencephalic nucleus; solid squares, jugular ganglion; solid circles, DRG; open diamonds, ventrolateral part of the trigeminal ganglion; open circles, geniculate ganglion; open triangles, petrosal ganglion; open squares, nodose ganglion.

1985) from chick embryos of 4, 5, 6, 8, 10, 12, and 14 d incubation (E4–E14). The maxillomandibular lobe of the trigeminal ganglion was further dissected into placode-derived and neural crest-derived components, the ventrolateral pole (VL-TG), and dorsomedial pole (DM-TG), respectively (Davies and Lumsden, 1983). In addition, at E3, the ganglia of placodal origin (the entire trigeminal ganglion at this stage, the geniculate, vestibular, petrosal, and nodose ganglia) were sufficiently well formed to permit dissection. The rostromedial part of the trigeminal mesencephalic nucleus (Davies, in press) was dissected from E10, E12, and E14 embryos. Paravertebral sympathetic and ciliary ganglia were dissected from E10 and E12 embryos.

Explant culture

Five explants of each type were embedded in 250 μ l of collagen gel (Lumsden and Davies, 1983) in 16-mm-diameter plastic wells (Nunc multiwell dishes) and overlaid with 60 μ l of heat-inactivated horse serum (Gibco) and 290 μ l of F14 medium (Barde et al., 1980). Control cultures and cultures supplemented with BDNF at concentrations of 25 and 100 ng/ml (unless otherwise stated) were set up at each age.

After 24 hr incubation at 37°C, the cultures were viewed by phase-contrast microscopy. The magnitude of neurite outgrowth from each explant was scored on an ordinal scale from 0 to 10 (Davies and Lumsden, 1984).

Dissociated neuron-enriched cultures

Vestibular ganglia and the DM-TG and VL-TG were trypsinized and dissociated as described previously (Davies and Lindsay, 1985). The dissociated cell suspension was enriched for neurons by the technique of differential sedimentation (Davies, in press). Neurons were grown in 35-mm-diameter plastic culture dishes (Nunc) at 2000–4000 neurons per dish. The substratum was prepared by sequentially coating dishes with polyornithine, 0.5 mg/ml (Sigma), and laminin, 10 μ g/ml (BRL), by the methods of Collins (1978) and Edgar et al. (1984), respectively. Neurons were grown in 2 ml of F14 medium containing heat-inactivated horse serum at 10%. Control cultures and cultures supplemented with either BDNF at 10 and 50 ng/ml or NGF at 5 and 25 ng/ml were set up in duplicate. After a 48 hr incubation, the number of neurons with neurites of lengths greater than five perikaryon diameters was counted. This number was expressed as the percentage of the number of cells plated.

BDNF purification

BDNF was purified from pig brain as described in Barde et al. (1982), with some modifications (Lindsay et al., 1985).

NGF purification

NGF was purified from adult male mouse submandibular salivary glands according to the method of Bocchini and Angeletti (1969), with the modifications described in Suda et al. (1978).

Results

Explant cultures

The magnitude of neurite outgrowth from explants grown in the absence of BDNF was, with the exception of the nodose and dorsal root ganglia, low at all ages. There was moderate neurite outgrowth from control cultures of E3 and E4 nodose ganglia and E8–E12 DRG (Fig. 1).

At E3 the magnitude of neurite outgrowth from explanted ganglia was not influenced by the presence of BDNF (Fig. 1). By E4 a number of ganglia had become responsive: The VL-TG and DRG showed a slight response, and neurite outgrowth from nodose ganglia in the presence of BDNF was significantly larger than that of controls (Mann-Whitney test, $p < 0.01$). By E8, all explants except the vestibular showed a clear response to BDNF; the geniculate and DM-TG explants were the last to respond. Trigeminal mesencephalic explants at E10, the earliest age at which this nucleus can be cleanly dissected from the midbrain, showed a marked response to BDNF. For all responsive ganglia, BDNF-promoted neurite outgrowth reached a maximum between E10 and E12 and declined between E12 and E14. Figure 2 summarizes the age-related trends in the response of explants to BDNF. The typical appearance of VL-TG and trigeminal mesencephalic explants grown with and without BDNF is shown in Figure 3.

Apart from a small number of neurites at E12 and E14, vestibular ganglion explants showed no response to BDNF throughout the period of development studied. To determine whether the lack of response of vestibular explants in the above series of experiments was due to the use of inappropriate concentrations of BDNF and/or a more stringent growth substratum requirement of vestibular neurites, E10 vestibular explants were incubated in the presence of BDNF at concentrations of 2, 5, 10, 40, 100, 200, 400, and 800 ng/ml with and without laminin (10 μ g/ml) incorporated into the collagen matrix. No neurite

Figure 3. Phase-contrast micrographs of E10 explants of the rostromedial part of the trigeminal mesencephalic nucleus (TMN) and ventrolateral pole of the trigeminal ganglion (VL-TG) after 24 hr in culture. *A*, VL-TG explant grown in control conditions; there is no neurite outgrowth. *B*, VL-TG explant grown in the presence of BDNF at a concentration of 100 ng/ml; a dense halo of neurites is visible. *C*, TMN explant grown in control conditions; no neurites can be seen. *D*, TMN explant grown in the presence of BDNF at 100 ng/ml, showing marked neurite outgrowth.

outgrowth was observed from vestibular explants under any of these conditions.

To determine the relationship between the onset of BDNF responsiveness and the onset of NGF responsiveness in populations of developing sensory neurons, we grew DRG explants of E4–E6 chick embryos with either BDNF or NGF. At E4 a slight response to BDNF was consistently observed in all experiments, but there was no response to NGF (Fig. 4). At E5 moderate neurite outgrowth was observed in response to both BDNF and NGF, and at E6 the magnitude of this response showed a further increase.

No fiber outgrowth was observed from explants of sympathetic and parasympathetic ganglia grown with BDNF.

Dissociated neuron cultures

In control cultures of E10 vestibular neurons, the majority of neurons degenerated: After 48 hr incubation, only $1.5 \pm 0.5\%$ (mean $\pm$ SEM, $n = 4$) of the neurons plated were growing. In the presence of BDNF at 50 ng/ml, however, $36.5 \pm 3\%$ of the neurons survived and grew after 48 hr incubation. The typical appearance of these neurons is shown in Figure 5.

In control cultures of E10 VL-TG and DM-TG neurons, fewer than 1% of the neurons survived 48 hr incubation. Whereas BDNF promoted the survival and growth of the majority of VL-TG neurons, only a small number (about 7%) of DM-TG neurons were supported. In contrast, NGF promoted the sur-

Figure 4. Bar chart showing the magnitude of neurite outgrowth from explants of E4–E6 DRG grown in the presence of either BDNF at 25 and 100 ng/ml (hatched bars) or of NGF at 1.25 and 5 ng/ml (stippled bars). Bar height, The median of neurite outgrowth scores after 24 hr incubation; upper and lower limits of the vertical line transecting each bar, the positive and negative interquartile deviations, respectively.

vival and growth of the majority of DM-TG neurons, but only about 5% of VL-TG responded. These differences in the response of VL-TG and DM-TG neurons to BDNF and NGF were also observed at E6, E8, and E12. In accordance with the results of explant cultures, the percentage survival was greatest in E10 cultures. The quantitative E10 data are shown in Figure 6, and the typical appearance of E10 DM-TG and VL-TG neurons grown with BDNF and with NGF is shown in Figure 7. Although cell counts were usually performed after 48 hr incubation, no significant changes were observed over the following days.

Discussion

We have shown that all populations of first-order sensory neurons contain BDNF-responsive neurons. In explant culture, BDNF elicited marked neurite outgrowth from the trigeminal mesencephalic nucleus and all sensory ganglia except the vestibular, and although unresponsive in explant culture, vestibular

Figure 6. Bar chart showing the percentage survival of E10 dorso-medial trigeminal (DM-TG) and ventrolateral trigeminal (VL-TG) neurons in control cultures (blank bars), cultures supplemented with NGF at 5 and 25 ng/ml (stippled bars), and cultures supplemented with BDNF at 10 and 50 ng/ml (hatched bars) after 48 hr incubation. Bar height, The mean, and line transecting the bars, the percentage survival in each of the duplicate cultures.

neurons were supported by BDNF in dissociated culture. The discrepancy in the action of BDNF on vestibular explants and on dissociated vestibular neurons may be due to the particularly prominent connective tissue capsule that closely invests this ganglion, hindering neurite outgrowth.

Explants differed in the age at which BDNF first elicited a response. The earliest to respond were the VL-TG, nodose, and DRG and the latest, the DM-TG, jugular and geniculate. The onset of the response, for most explants, appears to have been

Figure 5. Phase-contrast micrographs of dissociated neuron-enriched cultures of E10 vestibular neurons after 48 hr incubation. *A*, Control culture showing a degenerating and a phase-bright neuron without processes. *B*, Neurons growing in the presence of BDNF at 50 ng/ml.

Figure 7. Phase-contrast micrographs of dissociated neuron-enriched cultures of E10 ventrolateral trigeminal neurons (VL-TG) and dorsomedial trigeminal neurons (DM-TG) after 48 hr incubation. Control cultures and cultures supplemented with BDNF (50 ng/ml) and NGF (25 ng/ml) are shown.

related to the stage of development of the constituent neurons, that is, neurons generated earlier in development responded to BDNF before later generated neurons. For example, VL-TG and nodose neurons, which are generated between E2 and E5 (D'Amico-Martel, 1982; D'Amico-Martel and Noden, 1980), responded to BDNF earlier in development than DM-TG and jugular neurons, which are generated between E4 and E7. This relationship, however, does not seem to apply to either the geniculate ganglion, which responded late in development but whose neurons are generated at the same time as VL-TG and nodose neurons (D'Amico-Martel, 1982), or to DRG, which

responded early but whose neurons are generated at much the same time as DM-TG and jugular neurons (Carr and Simpson, 1978).

On the whole, sensory ganglia appeared to respond to BDNF during much the same stage of development as neural crest-derived sensory ganglia respond to NGF. The magnitude of the response to BDNF increased with age to reach a maximum between E10 and E12 (Fig. 2). Likewise, the response of neural crest-derived sensory ganglia to NGF shows a similar increase to reach a maximum between E8 and E11 (Davies and Lindsay, 1985). Furthermore, as has been shown here with DRG, the

onsets of the response to BDNF and NGF are closely related in development, occurring at E4 with BDNF and at E5 with NGF (Fig. 4). For the majority of sensory neurons, it is not precisely known whether the central and peripheral connections form during the same period of development or at different periods. Several detailed studies of vestibulo-acoustic neurons in the embryonic chick, however, have revealed that the central and peripheral synapses of these neurons form during approximately the same period, that is, during the second week of incubation (Whitehead and Morest, 1978, 1980). Should these features turn out to be shared by other sensory neurons, then our findings suggest that BDNF, like NGF, supports the survival of developing sensory neurons during the stage of target-field innervation.

The location of BDNF in the CNS suggests that this molecule provides trophic support to sensory neurons via their central rather than their peripheral branches. In this context it is interesting to note the recent demonstration by Yip and Johnson (1984) that central rhizotomy of neonatal DRG causes a 50% reduction in neuronal number, indicating that the survival of developing sensory neurons is indeed dependent on trophic support from the CNS.

Although our findings clearly show that BDNF-responsive neurons are present in all sensory ganglia and in the trigeminal mesencephalic nucleus, the results with dissociated neuron-enriched cultures of trigeminal neurons demonstrate that not all sensory neurons respond to BDNF. Whereas the majority of ventrolateral trigeminal neurons survived and grew in the presence of BDNF, only about 7% of dorsomedial neurons survived in cultures supplemented with this growth factor (Figs. 6 and 7). The low percentage of survival of DM-TG neurons was not due simply to an unsuitable culture environment, since the majority of these neurons survived and grew under similar conditions in the presence of NGF. This marked difference in the response of DM-TG and VL-TG neurons to BDNF was not apparent in explant cultures (see Fig. 1), which suggests that a small percentage of responsive neurons is enough to create a clear response from explants.

The present finding that NGF and BDNF have reciprocal effects on the survival of DM-TG and VL-TG neurons, together with the finding that NGF-responsive sensory neurons are restricted to neural crest-derived sensory ganglia (Davies and Lindsay, 1985), demonstrate that not all BDNF-responsive sensory neurons respond to NGF. Whether BDNF- and NGF-responsive sensory neurons comprise distinct subsets in neural crest-derived sensory ganglia is unclear. The finding that over 50% of cultured E6 DRG neurons survive and grow with saturating levels of either BDNF or NGF alone, although substantially more neurons respond in the presence of both factors (Lindsay et al., 1985), suggests that there is some partial overlap between BDNF- and NGF-responsive neurons.

The proposal that sensory neurons depend on the supply of both a factor from the periphery and a different factor from the CNS, based on the demonstration that cultured proprioceptive neurons are supported by both BDNF and a neurotrophic factor present in skeletal muscle (Davies et al., 1986), suggests that some sensory neurons (e.g., a proportion of DM-TG neurons) respond to a CNS-derived neurotrophic factor distinct from BDNF. Conversely, other neurons (e.g., VL-TG and nodose ganglion neurons) may respond to peripheral target-derived factors other than NGF. It will be interesting to see in future studies if it is possible to associate BDNF-responsiveness with certain functional types of sensory neurons.

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