Vol. 147, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 31, 1987 Pages 422-427

BINDING CONSTANTS OF SOLUBLE NGF-RECEPTORS IN RAT OLIGODENDROCYTES AND ASTROCYTES IN CULTURE

Dario Marchetti[1], Robert W. Stach[2], Russell Saneto[3], Jean de Vellis[3], and J. Regino Perez-Polo[1]

[1]Department of Human Biological Chemistry and Genetics, The University of Texas Medical Branch, Galveston, Texas 77550

[2]Department of Biochemistry, SUNY Upstate Medical Center, Syracuse, New York 13210

[3]Laboratory of Biomedical and Environmental Sciences, University of California, Los Angeles, Los Angeles, Ca. 90024

Received July 27, 1987

SUMMARY. The neuronotrophic factor NGF binds to peripheral neurons of the dorsal root ganglion and the sympathetic nervous system. NGF binds to a cell surface receptor, NGFR, on these cells and displays Kd's of 10^{-9} and 10^{-11}M. NGF receptors have also been reported for basal forebrain magnocellular neurons. In addition, NGF specifically binds to NGFR on Schwann cells although the biological significance of this binding is not known. Here we report that NGF binds in a saturable and specific fashion to receptors on cultured isolated populations of rat astrocytes but not to oligodendrocytes. The binding to astrocytes in culture displayed a Kd of 2.7 $\pm$ 1.0 nM with 36,000 receptors per cell.

There is ample evidence that NGF has a direct regulatory role on neuronal cell survival during development in the peripheral (PNS) and central nervous system (CNS) (1-2). In vitro, NGF has been shown to cause transformed human neuroblastoma (SK-N-SH-SY5Y) and rat pheochromocytoma (PC12) cells to become postmitotic and to stimulate a variety of metabolic processes (3). For example, NGF induces

Abbreviations: NGF = Nerve growth factor; DMEM = Dulbecco's Modified Eagle Medium; NP-40 = Noniodet-P40; PBS = Phosphate buffered saline.

0006-291X/87 $1.50

free radical scavenging enzymes in PC12 and SK-N-SH-SY5Y, a phenomenum that may be related to the role of NGF in the neuronal cell death associated with neuronal competition for targets of innervation in development (4).

However, the effects of NGF are not restricted to neurons. It has been demonstrated that there are receptors to NGF on Schwann cells and that NGF has mitogenic effects on embryonic neural crest precursor cells and certain subpopulations of monocytes (5,6,7). In addition, NGF has been shown to have stabilizing effects on lesioned spinal nerve tracts acting through dorsal root ganglia, in agreement with the hypothesis that NGF effects on central spinal neurons and tracts may be mediated via non-neuronal cells (8,9). In an effort to explore this hypothesis, we determined the extent and kind of soluble NGF receptors present in isolated populations of neonatal rat astrocytes and oligodendrocytes applying Scatchard analysis to binding of ^{125}I-NGF to solubilized receptor preparations. The results would suggest that isolated rat astrocytes, when in culture, display 10^4 NGF receptors/cell with equilibrium binding constants in the nanomolar range, not unlike those reported for Schwann cells, whereas oligodendrocytes did not demonstrate significant binding of NGF to a receptor in a saturable and specific fashion.

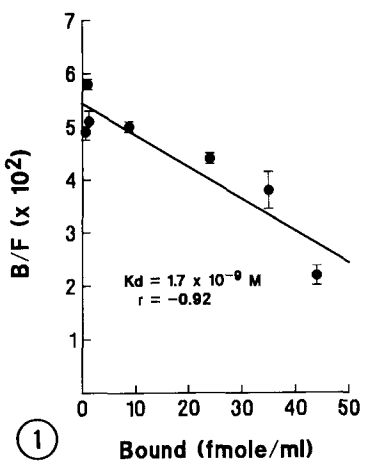
MATERIALS AND METHODS

Oligodendrocytes and astrocytes were isolated as described elsewhere (10). Upon isolation, astrocytes (type I) were grown to confluence in basal media containing 10% fetal calf serum, washed 3 x in basal media without serum and subsequently lots of 10^7 cells assayed for ability to bind ^{125}I-beta NGF. Oligodendrocyte progenitor cells were seeded in medium containing serum for 18 hrs and subsequently shifted to chemically defined medium (11) for a

five day period. At this time lots of 10 cells were assayed for ability to bind ^{125}I-beta NGF. Cells were solubilized in 0.5% NP-40 in PBS, pH 7.4 and incubated at 5° for 2 hours. The lysate was then centrifuged at 2000x g for 20 min. to remove nuclei and the recovered supernatant containing the solubilized receptor used in a receptor binding assay for solubilized NGF receptors. Murine beta-NGF was isolated as described elsewhere (12) and iodinated using the lactoperoxidase method (12). The specific activity was 2,000 cpm/fmole with 95% of the counts being trichloroacetic acid precipitable. NGF binding assays were carried out as described elsewhere (12). Aliquots were exposed to varying concentrations of ^{125}I-beta NGF in triplicate in the presence or absence of a 300 fold excess of beta NGF. Samples were allowed to equilibrate at room temperature for 2 hours and bound NGF then separated by precipitation of receptors in 21% polyethylene glycol as described elsewhere (12,13). Specific binding to NGF was calculated by subtracting non-specific binding from total binding. Non-specific binding was defined as that part of the total binding which remained in the presence of a 300 fold excess of unlabelled beta-NGF. The assays were performed in triplicate and linear regression analysis applied to Scatchard plots for determination of values. Three binding assays were carried out on different oligodendrocyte cultures on different occasions. Experiments on astrocytes were carried out four times on different lots of cells on different days.

RESULTS

When astrocytes were isolated from neonatal rat cerebral cortex primary cultures, they displayed $4.2 \pm 1.5 \times 10^4$ NGF receptors/cell with a Kd of $1.7 \pm 0.2 \times 10^{-9}$ M ($r = 0.92$; Figure 1). When this experiment was repeated three more times, the average value for four determinations was $3.6 \pm 1.6 \times 10^4$ NGF receptors/cell with an average K_d of $2.7 \pm 1.0 \times 10^{-9}$M. Alternatively, isolated oligodendrocytes did not display saturable or specific NGF binding ($r = 0.12$; Figure 2) as suggested by Scatchard analysis. In three separate experiments, using PC12 cells as positive controls, there was no significant specific binding (Figure 2) or the total binding values were less than or equal to the unspecific binding values (data not shown).



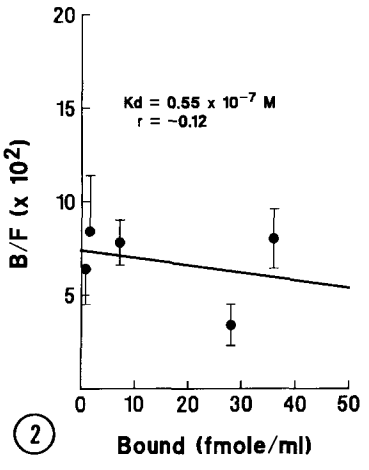


FIGURE 1. Scatchard analysis of soluble receptor binding assay data for NGF receptors on isolated cultured rat astrocytes.

FIGURE 2. Scatchard analysis of soluble receptor binding assay data for NGF receptors on isolated cultured rat oligodendrocytes.

DISCUSSION

Expression of NGF receptors by NGF-sensitive neurons in PNS involves two different binding affinities for NGF (13). The equilibrium binding constants for NGF receptors in PNS are in the 10^{-11}M and 10^{-9}M range respectively (13). For CNS, no determinations of binding constants are available except for the demonstration that there are NGF receptors present in discrete areas of brain and spinal cord (14). Interestingly, where the binding of NGF has been reported for non-neuronal cells, only one kind of binding activity with nanomolar Kd's has been found to be present (5,7,15). Given the known ability of astrocytes to behave as macrophages, as opposed to the behaviour of oligodendroglia that are more akin to Schwann cells, it is interesting that we could not demonstrate NGF binding by oligodendroglia but could do so by astrocytes. An alternative explanation would be that whereas some subpopulation of oligodendroglia

does have NGF receptors, it is a small component of the cells studied here or that this component is lost in our manipulations.

The expression of NGF receptors by astrocyte cells in culture does not necessarily imply that similar NGF binding activities are being expressed in vivo. It may be a consequence of the tissue dissociation and selective absorption techniques employed in separating astrocytes from oligodendrocytes or the culture conditions employed. However, the fact that under these conditions oligodendrocytes do not express NGF receptors would argue that NGF receptors in astrocytes are not an artifact although they may only be expressed under conditions where the astrocytes are proliferating as they are here.

Nevertheless, these conditions may be relevant to the study of lesioned CNS structures. In PNS, Schwann cells have been shown to display NGF receptors and DRG satellite cells take up NGF in vivo (9), presumably following binding to cell surface receptors. Since the rate of proliferation of astrocytes is much greater than that of oligodendrocytes, there may be a relationship between expression of the NGF receptor and rates of proliferation of non-neuronal cells. Thus, for astrocytes, as is the case for certain immunogenic and neural crest precursor cells (6,7), NGF may act as a mitogen in sharp contrast to its effect on neurons. Alternatively, the NGF binding referred to here may be related to the ability of astrocytes to express immunogenic properties in vivo as a consequence of injury to the CNS insofar as tissue culture paradigms are implicitly regeneration or recovery paradigms (4).

ACKNOWLEDGEMENTS. This work was supported in part by NINCDS grant NS18708 (JRP-P) and USPH HDHD-06576 (JV). Thanks to D. Masters for manuscript preparation.

REFERENCES

1. Cowan, W.M., Fawcett, J.W., O'Leary, D.O.M, Stanfield B (1984) Science 227:1258-1265.
2. Easter S.S., Purves D., Rakic P., Spitzer, N.C. (1985) Science 230:507-511.
3. Perez-Polo, J.R. and Haber, B. (1983) The Clinical Neurosciences 5,pp 37-51, Churchill-Livingston, Inc., New York.
4. Perez-Polo, J.R., and Werrbach-Perez, K. (1985) Recent Achievements in Restorative Neurology Upper Motor Neuron Functions and Dysfunctions 30, pp.321-337 Karger.
5. Zimmermann A., Sutter A. (1983) The Embo Journ. 2(6), 879-885.
6. Thorpe, L.W., and Perez-Polo, J.R. (1987) J. Neurosci. Res. in press.
7. Bernd, P. (1986) Devlopmental Biol. 115, 415-424.
8. Khan, T., Green, B., Perez-Polo, J.R. (1981) Abs. Soc. Neurosci. 7: 551
9. Khan, T., Green, B., and Perez-Polo, J.R. (1987) J. Neurosci. Res. in press.
10. McCarthy, K.D., and de Vellis, J. (1980) J. Cell Biol. 85, 890-902.
11. Saneto, R.P., and de Vellis, J. (1985) Proc. Natl. Acad. Sci. USA, 82, 3509-3513.
12. Lyons, C.R., Stach, R.W., and Perez-Polo, J.R. (1983) Biochem. Biophys. Res. Commun. 115, 1, 368-374.
13. Stach, R.W., and Perez-Polo, J.R. (1987) J. Neurosci. Res. 17, 1-10.
14. Johnson, E.M, Taniuchi, M., Clark, H.B., Springer, J.E., Koh S, Tayrien, MW. Loy R (1987) J. Neurosci. 7:923-929.
15. Thorpe, L.W., Stach R.W., Hashim G.A., Marchetti, D. and Perez-Polo, J.R. (1987) J. Neurosci. Res. 17, 128-134.