Interleukin-1 regulates synthesis of nerve growth factor in non-neuronal cells of rat sciatic nerve

Dan Lindholm, Rolf Heumann, Michael Meyer & Hans Thoenen

Department of Neurochemistry, Max-Planck-Institute for Psychiatry, D-8033 Planegg-Martinsried, FRG

The Schwann cells and fibroblast-like cells of the intact sciatic nerve of adult rats synthesize very little nerve growth factor (NGF) (ref. 1). After lesion, however, there is a dramatic increase in the amounts of both NGF-mRNA and NGF protein synthesized by the sciatic non-neuronal cells[1,2]. This local increase in NGF synthesis partially replaces the interrupted NGF supply from the periphery to the NGF-responsive sensory and sympathetic neurons, whose axons run within the sciatic nerve[1]. Macrophages, known to invade the site of nerve lesion during wallerian degeneration[3,4], are important in the regulation of NGF synthesis[5]. Here we demonstrate that the effect of macrophages on NGF-mRNA levels in cultured explants of sciatic nerve can be mimicked by conditioned media of activated macrophages, and that interleukin-1 is the responsible agent.

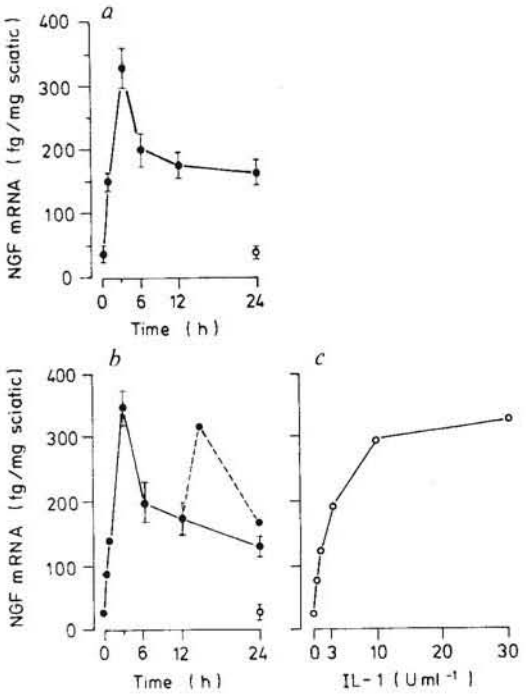

Fig. 1 *a*, Changes in NGF-mRNA levels of rat sciatic nerve segments by conditioned media of activated rat macrophages. *b*, Time course of induction by IL-1β. Human recombinant IL-1β (30 U ml^{-1}) of specific activity 4×10^4 U μg^{-1} protein was used. The dashed line shows the effect of re-addition of IL-1β to cultures incubated 12 h with the protein. *c*, IL-1β dose-response curve for NGF-mRNA induction. The incubation was for three hours and averages of two determinations at each concentration are given.
Methods. Sciatic nerve segments (3 cm) of adult Wistar rats were cultured in 1 ml Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. After three days culture, medium conditioned by either rat peritoneal macrophages or human recombinant IL-1β was added. Rat peritoneal macrophages were purified by attachment to plastic, and activated with lipopolysaccharide (LPS) (Sigma) for 20 h (70 μg ml^{-1}). Media conditioned by the adherent cells with more than 95% macrophages, as evaluated by the specific antibody ED1 (ref. 17), were collected and added to the nerve explant cultures. NGF-mRNA was determined by a quantitative Northern blot procedure, using a calibration standard (0.92 kilobases (kb)) for NGF-mRNA (ref. 16). The recovery of RNA was estimated using a shorter NGF-mRNA transcript (0.51 kb), which was added to the tissue samples before RNA extraction[16]. The autoradiograms were quantified on a scanning densitometer for the amount of NGF-mRNA present. Values are expressed as fg per mg wet weight of tissue, and they represent mean ±s.d. of three or more separate determinations. The open circles represent the control values of nerve segments incubated for 24 h.

In previous experiments we have shown that although the lesion-mediated increase in NGF-mRNA is biphasic *in vivo*[1], in cultures of rat sciatic nerve there was only a rapid and transient increase in NGF-mRNA, which was not followed by the chronic rise observed *in vivo*[5]. The addition of activated macrophages to the culture system resulted in increase of sciatic NGF-mRNA comparable to that observed *in vivo*[5]. To decide whether these effects result from cell-cell contact or are mediated by a humoral factor, we prepared media conditioned by activated rat macrophages. Figure 1 shows that rat sciatic nerve segments cultured for three days contained only relatively low amounts of NGF-mRNA which were markedly (11-fold) enhanced when the cultures were supplemented by macrophage-conditioned media, to reach a stable maximum after three hours. Of the many biologically active molecules synthesized by activated macrophages[6], prostaglandin E_2, and acidic and basic fibroblast growth factor (FGF) did not affect the amount of NGF-mRNA

Table 1 Effect of different agents on rat sciatic nerve NGF-mRNA transcription

	Sciatic NGF-mRNA (fg per mg tissue)
Controls	34.5
+PGE_2	47.2
+acidic FGF	28.0
+basic FGF	29.0
+PDGF	71.3
+TNF	75.2

Agents were added to sciatic nerve segments after culture for three days. After incubation for three hours, sciatic NGF-mRNA was quantitated as described in Fig. 1 legend. Prostaglandin E_2 (Sigma) was used at a final concentration of 5 μM (ref. 18); acidic and basic FGF (a gift from Dr Sensenbrenner[19]) were at a concentration of 5 ng ml^{-1}. PDGF (Speywood, UK) was used at a concentration of 3 U ml^{-1} (ref. 20). Human TNF-α (cachectin; Amgen, Amersham) was used at a final concentration of 1,000 U ml^{-1} (ref. 21). These concentrations are comparable to those known to produce a maximal effect. Values represent the average of two or more separate determinations.

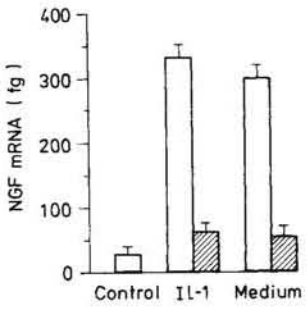

Fig. 2 Effect of macrophage-conditioned medium on sciatic NGF-mRNA; blockade by antibodies to IL-1. Conditioned media derived from LPS-activated human peritoneal macrophages were diluted 1 to 20 and used in the rat nerve explant cultures (see Fig. 1). A part of the medium was pretreated with rabbit antibodies raised against human IL-1 before adding it to the culture system. As a control 30 U of human recombinant IL-1 was treated similarly and then added to explant cultures. The antibodies used were polyvalent (Genzyme, Boston). Following incubation for three hours, the amounts of sciatic NGF-mRNA present were determined as described in Fig. 1 legend. The open bars show the results of incubating the nerve pieces with IL-1 or with the human macrophage-conditioned medium; the hatched bars represent results after pretreatment of the medium or of IL-1 with the specific antibodies. The values represent mean ±s.d. of three independent determinations.

produced (Table 1). Tumour necrosis factor (TNF) and platelet-derived growth factor (PDGF), which are major constituents of macrophage-conditioned media[7], doubled the amount of NGF-mRNA present. But the addition of recombinant human interleukin-1β (IL-1β) (ref. 8) resulted in a 14-fold increase in NGF-mRNA (Fig. 1*b*). The effect of IL-1β was discernible at a concentration of 1 U ml^{-1} and was maximal at about 10 U ml^{-1}. No difference was seen between the effects of recombinant IL-1α and IL-1β which are both produced by activated macrophages[9], supporting the view that these structurally different molecules (the homology between human and murine IL-1α and IL-1β is only 30%[10-12]) have similar biological activities. The effects of human IL-1 and rat macrophage-conditioned medium on NGF-mRNA are similar, both with respect to the extent and the time-course of the increase. Re-addition of IL-1β after 12 hours resulted in a repeat increase in NGF-mRNA to the level observed after the first addition, indicating that the protein was either not stable, or was degraded by the sciatic explants.

Because antibodies to rat IL-1 were not available, we used conditioned media of human macrophages, which affected the amounts of rat sciatic nerve NGF-mRNA produced to the same extent as the rat medium. The increase in NGF-mRNA observed after addition of recombinant IL-1 and human macrophage-derived medium could both be inhibited to the same extent by specific polyclonal antibodies to human IL-1 (Fig. 2). Thus, most, if not all, of the activity of the human macrophage-conditioned medium is due to IL-1. It is probable that the same is true for the conditioned medium of rat macrophages, although direct proof is not yet possible due to the unavailability of antibodies to rat IL-1 and to the poor interspecies immunological cross-reactivity[13]. Our interpretation is supported by the association of the activity of the rat-conditioned medium with fractions of relative molecular mass (M_r) between 10,000-20,000 (10-20K) (data not shown).

These results could explain the *in vivo* and the *in vitro* discrepancy[5]: *in vitro*, macrophages must be added to simulate the chronic increase in NGF-mRNA which occurs after lesion *in vivo*, and which remains high for more than two weeks[1,5]. Following a peripheral nerve injury, macrophages invade the site of lesion[3,4] to stimulate concomitant increase in NGF-mRNA levels[5]. These observations combined with ours indicate that macrophages are responsible for the persistent increase in NGF in the injured nerve *in vivo*. Note that macrophages themselves synthesize very little NGF (ref. 5), therefore their contribution to the amount of NGF present is indirect and mediated mainly by IL-1.

Macrophages and the IL-1 they release could also be important in the regulation of NGF synthesis during normal development; the amount of NGF-mRNA in the sciatic nerve of newborn rats is comparable to that in lesioned adult nerves[5], and decreases to adult values by the third postnatal week[5], by which time the high density of macrophages in the newborn rat sciatic nerve has also decreased to the low intact adult nerve levels[4]. Interestingly, IL-1 activity has also been detected in the central nervous system following brain injury[14], but cellular site of synthesis has not yet been established[13,15].

We thank Dr A. Shaw (Biogen) and A. Gronenborn (Martinsried) for a gift of human recombinant IL-1. We are also grateful to Dr M. Blumenstein for help with obtaining human macrophages, to Elfi Grossmann for typing the manuscript, and to Drs D. Edgar and Y.-A. Barde for critically reviewing the manuscript. D.L. is a recipient of a fellowship from EMBO.

Received 17 August; accepted 20 October 1987.

1. Heumann, R., Korsching, S., Bandtlow, C. & Thoenen, H. *J. cell Biol.* **104,** 1623-1631 (1987).
2. Bandtlow, C. E., Heumann, R., Schwab, M. E. & Thoenen, H. *EMBO J.* **6,** 891-899 (1987).
3. Perry, V. H., Brown, M. C. & Gordon, S. *J. exp. Med.* **165,** 1218-1223 (1987).
4. Stoll, G. & Müller, H. W. *Neurosci. Lett.* **72,** 233-238 (1986).
5. Heumann, R. *J. exp. Biol.* **132,** 133-150 (1987).
6. Nathan, C. F., Murray, H. W. & Cohn, Z A. *New Engl. J. Med.* **303,** 622-626 (1980).
7. Shimokado, K. *et al. Cell* **43,** 277-286 (1985).
8. Wingfield, P. *et al. Eur. J. Biochem.* **160,** 491-497 (1986).
9. Oppenheim, J. J., Kovacs, E. J., Matsushima, K. & Durum, S. K. *Immun. Today* **7,** 45-56 (1986).
10. March, C. J. *et al. Nature* **315,** 641-647 (1985).
11. Lomedico, P. T. *et al. Nature* **312,** 458-461 (1984).
12. Auron, P. E. *et al. Proc. natn. Acad. Sci. U.S.A.* **81,** 7907-7911 (1984).
13. Giulian, D., Baker, T. J., Shih, L.-C. N. & Lachman, L. B. *J. exp. Med.* **164,** 594-604 (1986).
14. Giulian, D. & Lachman, L. B. *Science* **228,** 497-499 (1985).
15. Fontana, A., Kristensen, F., Dubs, R., Gemsa, D. & Weber, E. *J. Immun.* **129,** 2413-2419 (1982).
16. Heumann, R. & Thoenen, H. *J. biol. Chem.* **261,** 9246-9249 (1986).
17. Dijkstra, C. D., Dopp, E. A., Joling, P. & Kraal, G. *Immunology* **54,** 589-599 (1985).
18. Brandwein, S. R. *J. biol. Chem.* **261,** 8624-8632 (1986).
19. Pettman, B., Labourdette, G., Weibel, M. & Sensenbrenner, M. *Neurosci. Lett.* **68,** 175-180 (1986).
20. Grennberg, M. E. & Ziff, E. B. *Nature* **311,** 433-438 (1984).
21. Bevilacqua, M. P. *et al. Proc. natn. Acad. Sci. U.S.A.* **83,** 4533-4537 (1986).