Regional and Cellular Codistribution of Interleukin 1β and Nerve Growth Factor mRNA in the Adult Rat Brain: Possible Relationship to the Regulation of Nerve Growth Factor Synthesis

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Abstract. We have found a regional distribution of IL 1β mRNA and IL 1 activity in the normal adult rat brain, which reveals at least partially a colocalization with nerve growth factor (NGF). The predominantly neuronal signal patterns were found over the granule cells of the dentate gyrus, the pyramidal cells of the hippocampus, the granule cells of the cerebellum, the granule and periglomerular cells of the olfactory bulb, and over dispersed cells of the ventromedial hypothalamus and of the frontal cortex. In these areas also the highest levels of IL 1 activity were observed. In the striatum and septum much lower levels of IL 1β mRNA and IL 1 activity (shown for the striatum), most likely synthesized by glial cells, could be determined. IL 1β-expressing cells were mainly found in brain regions that also synthesize NGF mRNA as shown by in situ hybridization. NGF mRNA could be demonstrated over pyramidal cells of the hippocampus, granule cells of the dentate gyrus, periglomerular cells of the olfactory bulb and over prefrontal cortex neurons. These data indicate that IL 1β, among other factors, might also play a regulatory role in the synthesis of NGF in the CNS, as has been demonstrated in the peripheral nervous system (Lindholm, D., R. Heumann, M. Meyer, and H. Thoenen. 1987. *Nature (Lond.).* 330:658–659).

THE physiological function of nerve growth factor (NGF)[1] for specific populations of neurons of the peripheral and central nervous systems is well documented. NGF is essential for the development and maintenance of function of the peripheral sympathetic and neural crest–derived sensory neurons (see Levi-Montalcini and Angeletti, 1968; Thoenen and Barde, 1980; Green and Shooter, 1980). In the central nervous system a similar function of NGF has recently been established for cholinergic neurons of the basal forebrain nuclei (see Thoenen et al., 1987; Whittemore and Seiger, 1987). Whereas the function of NGF as a retrograde messenger transferring information from the field of innervation to the NGF-responsive innervating neurons was unambiguously established, very little is known about the mechanisms involved in the regionally differential regulation of NGF synthesis. In the peripheral nervous system, we have recently demonstrated that lesioning of the sciatic nerve leads to a dramatic increase in NGF synthesis by nonneuronal cells (Heumann et al., 1987 *a*,*b*). It could also be demonstrated that at least the long-lasting augmentation of NGF synthesis is mediated by immigrating macrophages. Of the many cytokines synthesized and released by macrophages IL 1 was shown to be the predominantly responsible agent for the regulation of the reactive synthesis of NGF (Lindholm et al., 1987, 1988). Interestingly, IL 1-like activity has also been found in the normal rat brain (Nieto-Sampedro and Berman, 1987), which increases after injury. However, the site of synthesis and the physiological functions are unknown. More recently, we have demonstrated that IL-1 induces the synthesis of NGF in astrocytes in vitro and increases also the NGF mRNA levels in hippocampus after intraventricular injections (Spranger et al., 1990). These results suggest that also in the CNS IL-1 has the potential to regulate NGF synthesis. However, from these experiments it could not be deduced whether IL 1 is also involved in the physiological regulation of NGF synthesis.

We now demonstrate that IL 1β mRNA and IL 1 activity are partially colocalized with NGF mRNA in certain brain regions of the normal adult rat. In situ hybridization experiments show a predominantly neuronal expression of both the IL 1β mRNA and the NGF mRNA, which indicates that IL 1β might indeed play a functional role in the regulation of NGF synthesis in the CNS.

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1. *Abbreviation used in this paper*: NGF, nerve growth factor.

Materials and Methods

Cell Culture

Primary Brain Cultures. Cerebri of newborn rats were processed by the method of McCarthy and DeVellis (1980). Cells were seeded in 75-cm Falcon flasks and cultivated in DME medium (Gibco Laboratories, Paisley, Scotland) with 10% FCS (Gibco Laboratories) for 2 wk at 10% CO_2. The medium was changed every fourth day. The cell monolayer was taken off with 0.1% trypsin/5 mM EDTA (Sigma Chemical Co., St. Louis, MO), triturated, and replated on poly-L-lysine (Sigma Chemical Co.) coated four-well Greiner dishes with 10,000 cells/well. 2 d before use for in situ hybridization the cells were switched to serumless DME medium (Spranger et al., 1990).

Cerebellar Granule Cells. Granule cells were prepared from cerebella of 7-d-old rats by trypsin treatment (0.1% [vol/vol], 15 min; Balazs et al., 1988; Hatten et al., 1988). The cells were cultured for 3 d on poly-L-lysine-coated dishes (5 μg/ml) in a medium consisting of one part of Eagle's basal medium with Earle's salts (Gibco Laboratories), one part of Ham's F12 medium with 33 mM glucose and 2 mM glutamine and the following supplements: 25 μg/ml insulin, 100 μg/ml transferrin, 20 nM progesterone, 100 μM putrescine, 30 nM selenium (Bottenstein and Sato, 1979). Cytosine arabinoside (10 μM) was routinely added to the cultures to inhibit the growth of nonneuronal cells. Granule cells were identified by their small size and by the extensive outgrowth of neurites during the first days of culture (Balazs et al., 1988; Hatten et al., 1988).

Activated Macrophages. Activated rat peritoneal macrophages were obtained as described by Lindholm et al. (1988) and Heumann et al. (1987*b*).

Raising of Polyclonal Rat IL 1β Antibodies

The NH_2-terminal peptide of mature rat IL 1β (amino acids 117–143 according to Nishida et al. [1988] with an additional tyrosine at position 144) was synthesized on a peptide synthesizer (model 431A; Applied Biosystems, Foster City, CA) using Fmoc chemistry. New Zealand White Rabbits were immunized with native, uncoupled peptide by standard protocols. In ELISA tests using the peptide adsorbed to plastic as a solid phase (Flanders et al., 1988) the serum had a titer of 10^{-4} after the third booster injection.

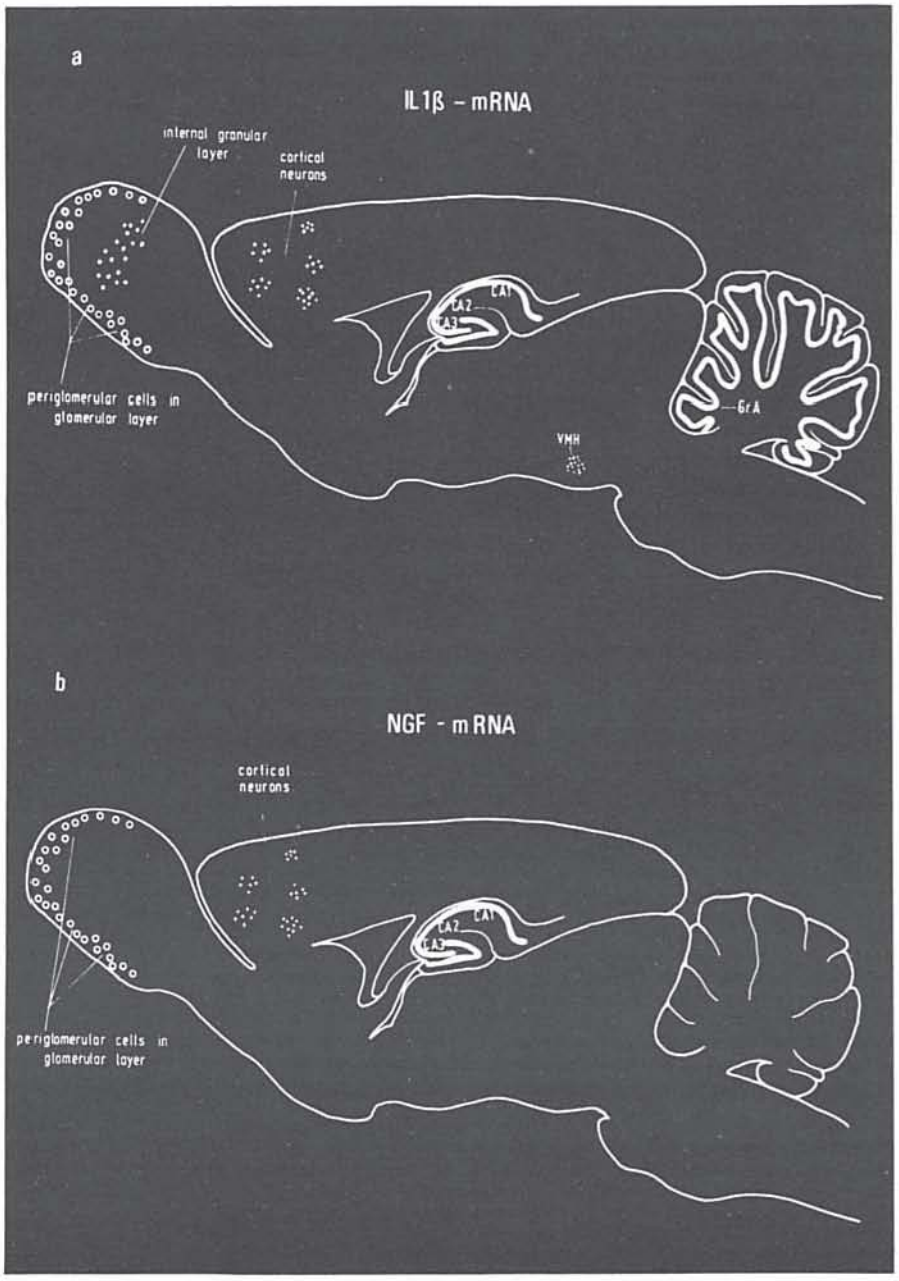

Figure 1. Schematic presentations of the localization of IL-1β mRNA (*a*) and NGF mRNA (*b*) in the adult rat brain. The sites of synthesis are dotted and represent the hippocampus (CA1–CA3), cerebellar granule cells (*GrA*), granule and periglomerular cells of the olfactory bulb, neurons in the frontal cortex, and neurons of the ventromedial hypothalamus (*VMH*). Not shown are hybridizations of IL 1β mRNA in the striatum and septum. Abbreviations and drawings after Pellegrino et al. (1979).

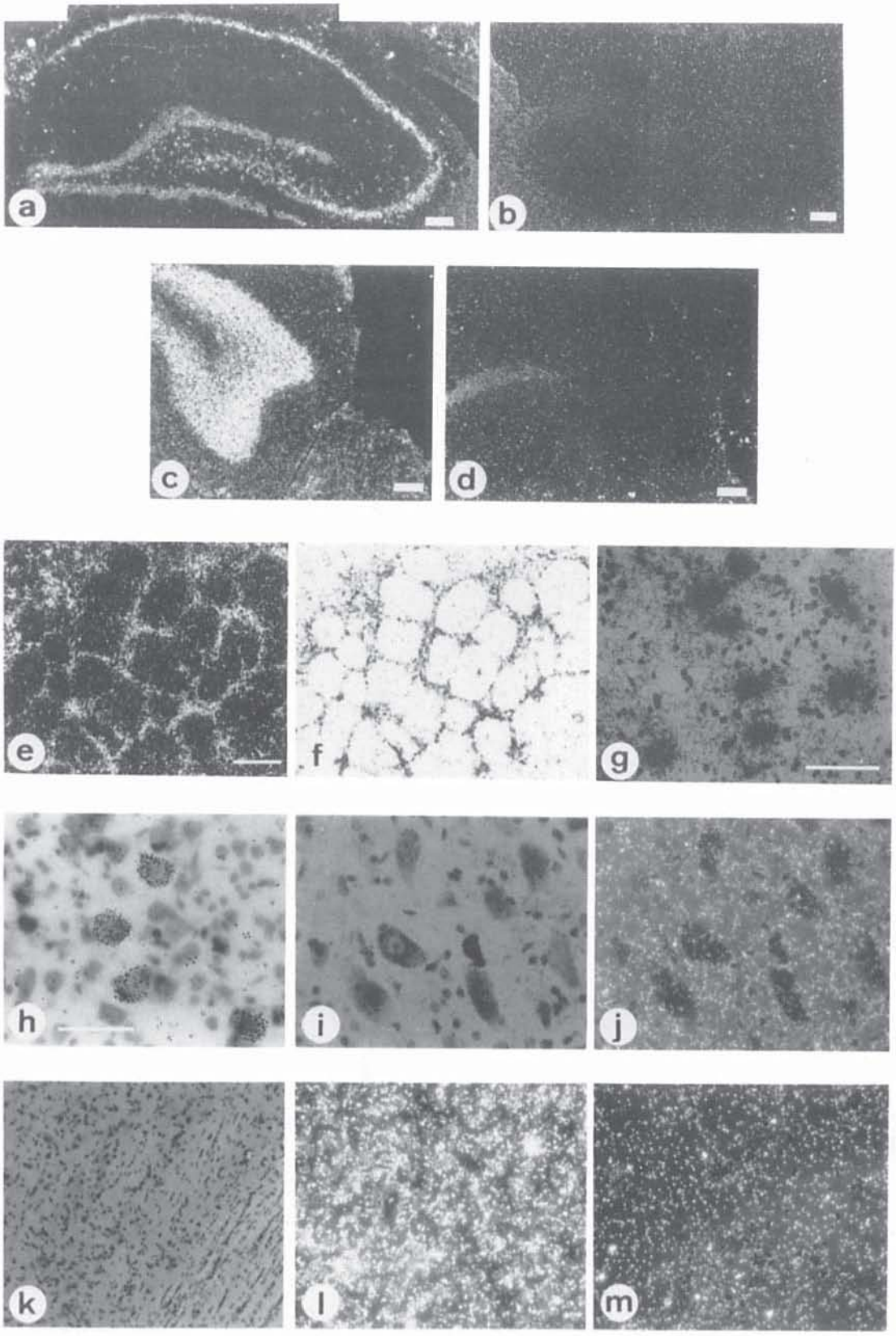

Figure 2. Results from in situ hybridization of rat brain sagittal sections to IL 1/40 riboprobes. Positive IL 1β hybridizations are shown for the hippocampus (*a*), the granule cells of the cerebellum (*c*), periglomerular cells of the olfactory bulb (*e*) with the corresponding phase contrast photomicrograph (*f*), neurons of the hypothalamus (*g*), and neurons of the frontal cortex (*h*). No specific labeling is seen on sections hybridized with the sense IL 1/40$^-$ probe (*b* and *d*). Positive labeling is also shown over glial cells in the septum (*j*) and striatum (*l*) with the corresponding phase contrast pictures (*i* and *k*). Background labeling is shown of a section through the striatum hybridized with the sense IL 1/40$^-$ riboprobe (*m*). All sections were counterstained with cresyl-violet. Exposure time was 5.5 wk at 4°C. Bars, (*a–d*) 1 mm; (*e–m*) 50 μm.

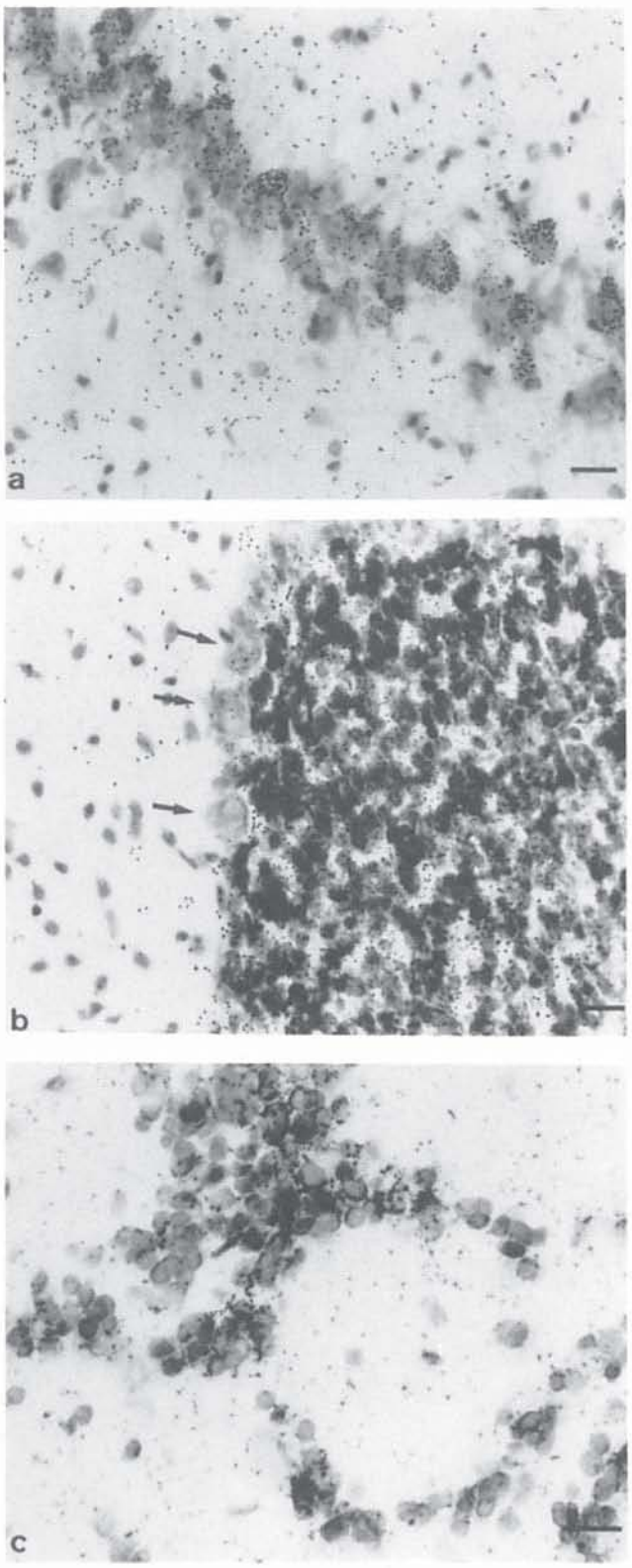

Figure 3. Higher magnifications of the positive IL 1β mRNA hybridization signals found over granule cells of the dentate gyrus (*a*), the cerebellum (*b*), and the periglomerular cells of the olfactory bulb (*c*), indicating a differential expression of the IL 1β mRNA. Note, that the Purkinje cells (*arrows*) in the cerebellum are not labeled (*b*). Exposure time was 4 wk at 4°C. Bar, 100 μm.

Costimulator Assay for IL 1 Activity

After transcardial perfusion of adult Wistar rats (180–200 *g*) with Dulbecco's modified Eagle's medium (Sigma Chemical Co.), brains were rapidly dissected. Corresponding brain regions of each animal were pooled and stored at −70°C. Samples were homogenized in 500 μl/100 mg wet weight of buffer H (50 mM NaCl, 10 mM Hepes, 1 mM EDTA 20 μM 2-mercaptoethanol, 0.1 mM PMSF, 2 μg/ml Pepstatin A, pH 7.0) by 10 strokes in a glass Teflon homogenizer. After removal of membranes by sequential low-speed (12,000 *g*, 5 min, 4°C) and high-speed centrifugation (TLA 100.3 rotor; 50,000 rpm, 15 min, 4°C, Beckman Instruments, Fullerton, CA), samples were analyzed by FPLC using a Superose 12 column (HR 10/30, Pharmacia Fine Chemicals, Piscataway, NJ). Elution was performed with 150 mM NaCl, 50 mM Na_2HPO_4, pH 7.4, collecting 500 μl fractions. Myoglobin and lysozyme (Sigma Chemical Co.) were used as molecular weight standards. IL 1 activity was assessed for each fraction at a one-tenth dilution as described by LeMoal et al. (1988) using the EL4 cell clone as indicator of IL 2 receptor expression. The assay was modified to a three-step procedure applying biotinylated second antibodies and streptavidin-coupled peroxidase (Amersham Chemicals). The sensitivity limit was $\sim 10^{-6}$ U per assay of human recombinant IL 1β (Biogen, Geneva, Switzerland) used as an internal standard. As an additional positive control dilutions of conditioned medium from activated rat macrophages were assayed. Neither NGF (up to 50 ng/ml) nor IL-6 (up to 200 U/ml) were active in this assay. Analysis by the described method of 1 U of recombinant human IL 1β in 5% FCS resulted in 70–85% recovery of activity. To exclude specific differences in inhibition or degradation of endogenous IL 1 activity, samples of brain regions were homogenized after addition of recombinant IL 1β in 10-fold excess over the average of endogenous IL 1 activity. In all cases the recovery was 70%.

All experiments for immunoadsorption were done with an IgG fraction (called B-IgG) prepared by protein A-Sepharose chromatography from the bleeding after the third boost. The immunoadsorption columns contained 50 μl of packed preswollen protein A-Sepharose. 100 μg of B-IgG or, for control columns, the same amount of commercial rabbit IgG (Miles Laboratories, Naperville, IN) in 600 μl of 150 mM Tris, pH 8.8 were passed twice over the column. For immunoadsorption the column was washed with 1 ml of culture medium used for EL-4 cells (RPMI 1640, 5% FCS, 20 μM 2-mercaptoethanol, 12 mM Hepes; R-medium). 50 μl of the most active fraction from the preceding Superose 12 chromatography step were passed over the immunoadsorption or the control column. Thereafter the column was washed twice with 50 μl of R-medium and the flow through as well as the washes were pooled and applied on a second column of the same type. The flowthrough and the washes of this column were pooled and tested at a one-fifth dilution in the EL-4 costimulator assay.

In Situ Hybridization

Adult Wistar rats (180–200 g) were ether anaethesized and transcardially perfused with prewarmed 4% paraformaldehyde/PBS (Fluka AG; Buchs, Switzerland) for 30 min. Brains were removed, immersed in the fixative for 2 h, before being incubated in 15% sucrose/PBS overnight at 4°C. Brains were then frozen and 10-μm sagittal sections were collected on (50 μg/ml; Sigma Chemical Co.) coated poly-L-lysine glass slides. Cultures were treated with the same fixative for 30 min before thorough washing with PBS. Slides and cultures were further processed as described by Bandtlow et al. (1987), including an acetylation step before prehybridization (Hayashi et al., 1978).

Conditions for IL 1β-specific Probes. To get IL 1β-specific probes of a defined length and sequence a synthetic oligonucleotide of 40 bases (base 777–816 of the mouse IL 1β cDNA; Gray et al. [1986]) was subcloned into the Bluescript vector (Promega Biotech, Madison, WI). The sequence for the oligonucleotide was selected by the following criteria: (*a*) low homology to mouse IL 1α to ensure specific hybridization to IL 1β; (*b*) high homology to human IL-1β to increase the probability that mouse and rat IL 1β is detected by the probe; and (*c*) low homology to 28S ribosomal RNA to reduce nonspecific binding. In vitro ^{35}S-labeled sense (IL:-1β/40$^-$) and antisense (IL-1β/40$^+$) transcripts of linearized plasmids were made as recommended by the manufacturer (Promega Biotec). In comparison, we also used ^{35}S-labeled single-strand DNA probes obtained by reverse transcription of non-labeled sense and antisense transcripts. These probes resulted in a similar specific activity of 8×10^8 cpm/μg and were used under the same conditions as for cRNA probes, with the exception that the RNase A digestion step was omitted. Single stranded RNA or DNA probes were diluted to 30,000 cpm/μl in the following hybridization buffer: 50% formamide,

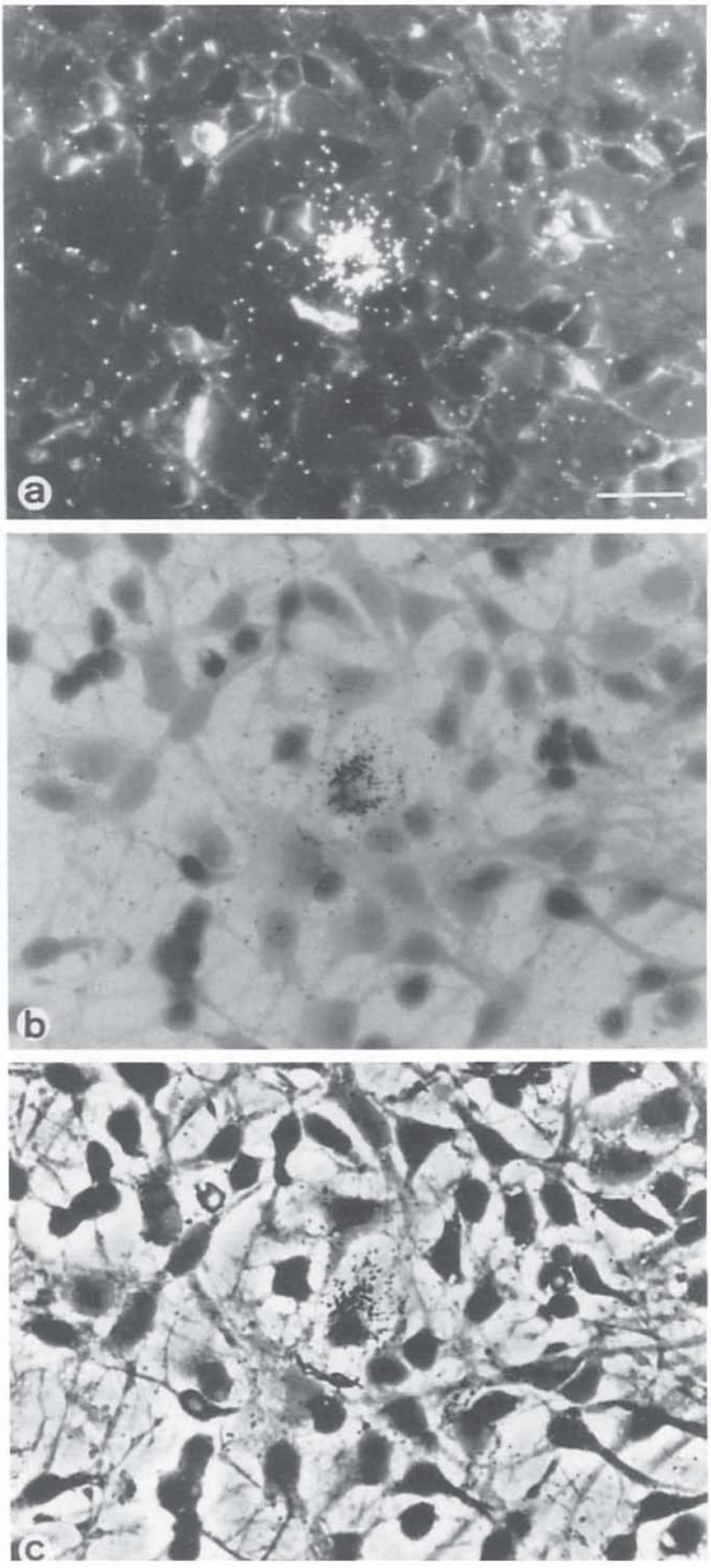

Figure 4. Localization of IL 1β mRNA in primary brain cultures of newborn rats after 2 d in vitro under low serum conditions (0.5% FCS). Dark field (*a*), the corresponding bright field (*b*), and phase-contrast (*c*) photomicrographs showing a selective labeling of ~5% of the cells.

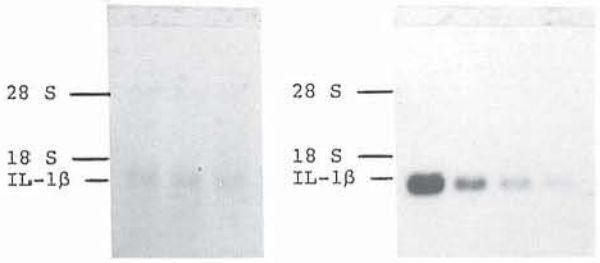

Figure 5. Northern blot of total RNA from purified cerebellar granule cells and of RNA from activated rat macrophages, hybridized with the ^{32}P-labeled IL 1β/450 riboprobe. Cells had been in culture for 4 d; RNA was extracted and processed as described in Materials and Methods. Positions of the ribosomal 18 and 28S RNA are indicated. The filters were RNase-treated and the exposure time for neuronal RNA (*left*) was four times longer than that of activated macrophages (*right*). Roughly 5 μg total neuronal RNA was loaded in all lanes, whereas total macrophage RNA was applied to a two-time dilution series of 5 μg.

0.6 M NaCl, 10 mM Tris, pH 7.0, 0.05% yeast total RNA (Sigma Chemical Co.), 2× Denhardt's, 0.05% inorganic sodium pyrophosphate, 10 mM methionine, 10 μM nonlabeled thio-UTP (New England Nuclear, Dreieich, FRG), 20 mM β-mercaptoethanol. Prehybridization (2 h) and hybridization (12 h) were carried out at 45°C. Thereafter, cultures and slides were rinsed several times with 2× SSC and washed three times for 30 min in 0.2× SSC at 40°C. Cells and sections were then treated with RNase A (15 μg/ml in 0.5 M NaCl, 10 mM Tris, pH 8, 1 mM EDTA) at 37°C for 30 min, followed by a wash in RNase buffer for 15 min, before they were finally washed in 0.1× SSC for 15 min at 55°C. Culture dishes and slides were then dehydrated, air dried, dipped in Kodak NTB 3 emulsion and exposed at 4°C. Autoradiographs were counterstained with cresyl violet after development.

Conditions for NGF-specific probes. A fragment of 118 bp from the coding region of the mouse NGF cDNA (amino acid 188–227; Scott et al., 1983) was subcloned into the Bluescript vector (Promega Biotec). Nonlabeled sense and antisense transcripts were reverse transcribed in the presence of ^{35}S-labeled dGTP. Hybridization was carried out in the above described buffer at 50°C. Washing steps were performed three times in 0.2× SSC at 50°C for 30 min and finally in 0.1× SSC, 25% formamide at 50°C. Slides were further processed as described above.

Northern Blot Analysis

Total cellular RNA from cultured cerebellar granule cells was isolated and further processed for Northern blot analysis as described by Lindholm et al. (1988). Briefly,RNA was glyoxylated and electrophoresed through a 1.2% agarose gel. After transfer of RNA to nylon membranes (Hybond; Amersham Chemicals) hybridization was carried out at 60°C in the presence of 50% formamide as described previously (Heumann et al., 1987*a*). The IL 1 clone used for Northern blots was prepared by the polymerase chain reaction (PCR) using oligonucleotides constructed against two regions of the mouse IL-1β cDNA (amino acids 53–58 and 207–213 for the sense and antisense oligonucleotides, respectively) (Young and Sylvester, 1989). The cDNA used was obtained by reverse transcription of RNA from activated rat macrophages. The rat IL 1β clone (450 bp) was further subcloned into the bluescript vector and sequenced and ^{32}P-labeled cRNA run-off transcripts were used at a concentration of 2 × 10^6 cpm/ml for Northern blot hybridization. After hybridization the filters were washed at 55°C in 2× SSC for 30 min and exposed to x-ray films. The specificity of the rat IL 1β probe was shown by RNase A treatment of the Northern blots and by comparison with RNA from activated macrophages.

Results

In Situ Hybridization

To determine the cellular localization of the specific expression of the IL 1β and NGF mRNA, sagittal sections of adult rat brains were hybridized with ^{35}S-labeled antisense DNA$^-$ or cRNA$^-$ probes (IL 1β/40$^+$; NGF/118$^+$). Control sections were hybridized under the same conditions with ^{35}S-labeled sense DNA$^-$ or cRNA$^-$ probes (IL 1β/40$^-$; NGF/118$^-$). The autoradiographic results are summarized in Fig. 1, by schematic presentations of the sites of IL 1β and NGF expression.

Localization of IL 1β mRNA

Cell bodies hybridizing to the IL 1β/40$^+$ probe were found in the hippocampal pyramidal cell layer, the granule cells of the dentate gyrus (Fig. 2, *a* and *b*), the granule cells of the cerebellum (Fig. 2, *c* and *d*), the granule and periglomerular cells of the olfactory bulb (Fig. 2, *e* and *f*), disperse cells of the anterior hypothalamus (Fig. 2 *g*), and of the frontal cortex (Fig. 2 *h*). A higher magnification of the labeling pattern in the dentate gyrus suggests a differential expression of the IL 1β mRNA since not all cell bodies of the granule cells are labeled (Fig. 3 *a*). The same is even more evident for the periglomerular cells of the olfactory bulb, where the signal is present only over a few cells (Fig. 3 *c*). In contrast, in the cerebellum, where exclusively granule cells were labeled, the grain distribution was intense and equal (Fig. 3 *b*). A consistent but much weaker labeling was detected over the striatum and septum (Fig. 2, *i–m*). However, in these brain areas the grains were homogenously distributed in between the neurons which are spared, suggesting that most probably glial cells are responsible for the signal. In comparison, no labeling occurred in brain regions rich in oligodendrocytes such as the corpus callosum (data not shown), indicating that oligodendrocytes do not contribute to the specific IL 1β signal in situ. These results are consistent with in situ hybridization experiments done on unstimulated primary cultures of newborn rat brains. In these cultures, consisting of oligodendrocytes, astrocytes, fibroblasts and microglia cells, only a very small number of cells (~5%) express IL 1β under serum free conditions (Fig. 4). Although it was not possible to identify the labeled cells unequivocally, they could well present amoeboid microglia cells since the number of labeled cells correlated with the number of microglia cells found under these culture conditions by immunocytochemical staining (Spranger et al., 1990). The neuronal identity of IL 1β-containing cells was further assessed by Northern blot analysis of cultured cerebellar granule cells from 7-d-old rats. These purified cells produce a typical 1.6–1.7 kb IL 1β transcript (Fig. 5) as described for macrophages (Giulian et al., 1986). However, as demonstrated in Fig. 5, the cerebellar granule cells contain a much lower copy number of IL 1β as compared with activated macrophages. The presence of IL 1β in cultured cerebellar granule cells was additionally confirmed by in situ hybridization as shown in Fig. 6, and reflects the sensitivity of the detection system.

Localization of NGF mRNA

Similar labeling patterns as described for IL 1β were found for the distribution of NGF mRNA. As has been shown by others (Rennert and Heinrich, 1986; Ayer-Le Lièvre et al., 1988; Whittemore et al., 1988) labeled cell bodies were found in the pyramidal cell layer of the hippocampus and in the granule cell layer of the dentate gyrus (Fig. 7 *a*). Interestingly, in this particular region the labeling signal found for NGF strikingly resembles the pattern seen for IL 1β (see Fig. 2 *a*), suggesting that both messages might be synthe-

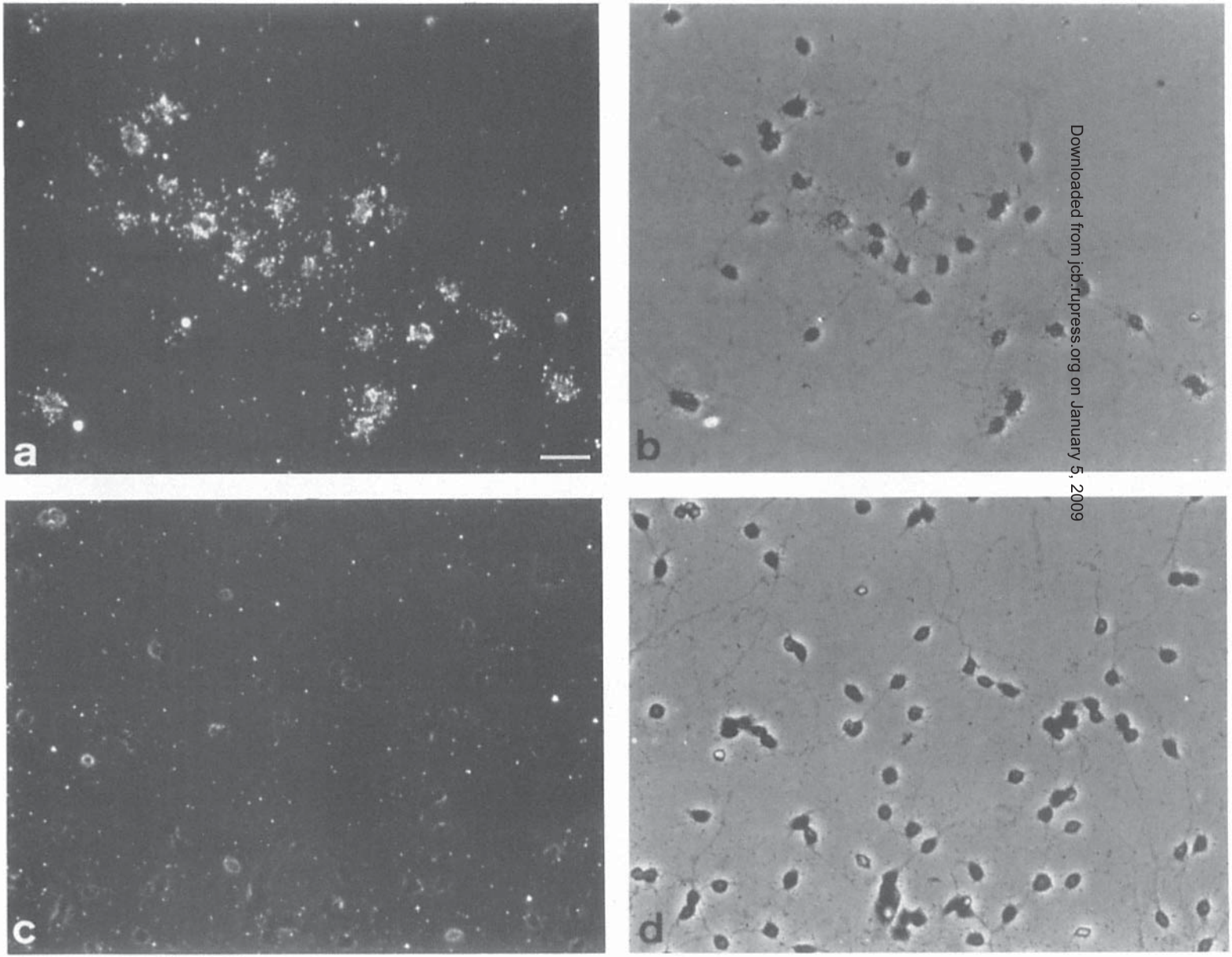

Figure 6. Localization of IL 1β mRNA in cerebellar granule cells from 7-d-old rats after 2 d in culture. Darkfield photomicrograph and corresponding phase contrast picture of granule cells hybridized with the IL $1\beta/40^{+}$ single-stranded DNA probe (*a* and *b*). No signal is seen over cells, hybridized with the sense probe IL $1\beta/40^{-}$ (*c* and *d*). Exposure time was 4 wk. Bar, 100 μm.

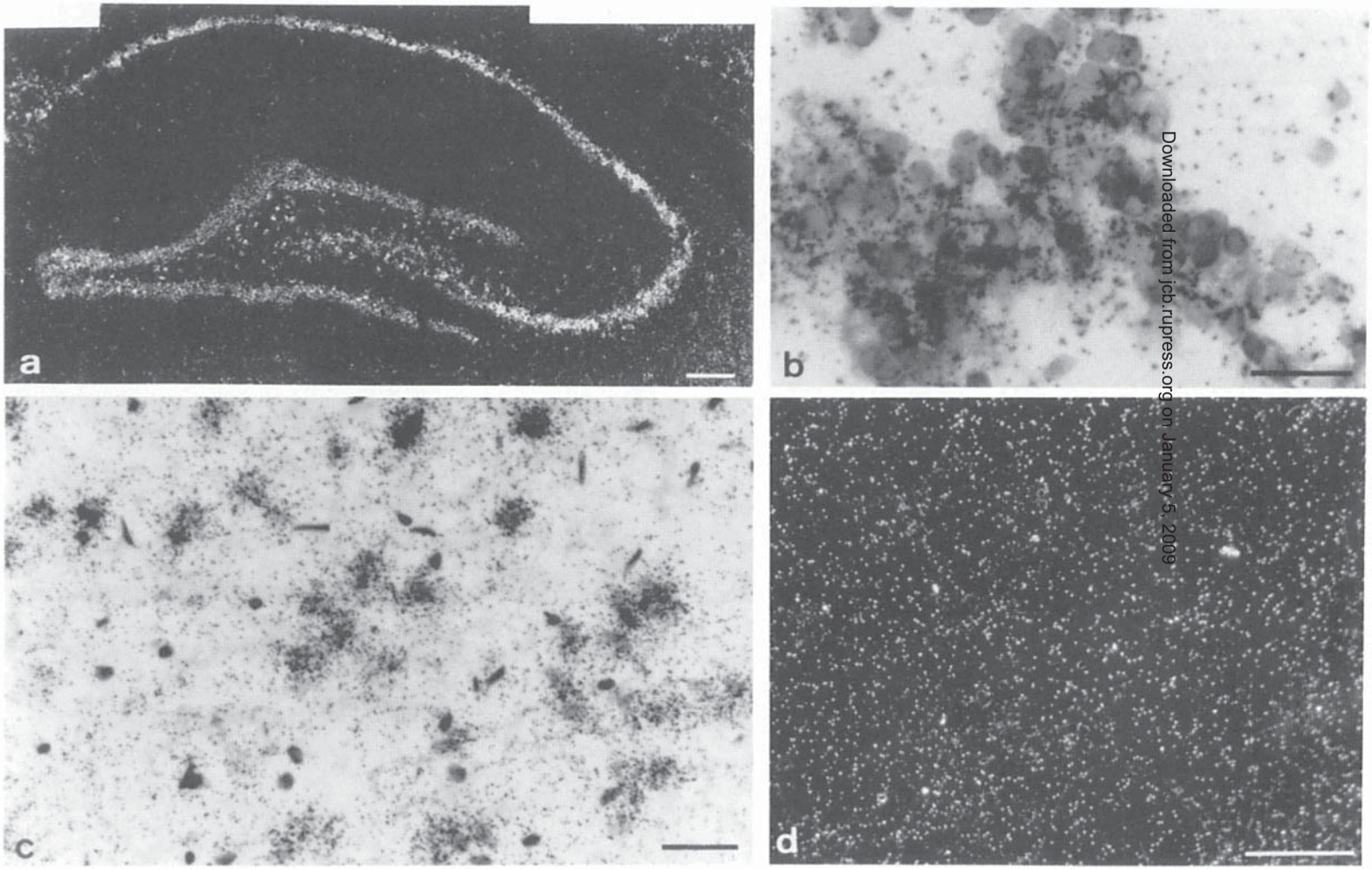

Figure 7. Results from in situ hybridization from rat brain sections hybridized with NGF/118 single stranded DNA probes. Positive signals are shown for the hippocampus (*a*), the periglomerular cells of the olfactory bulb (*b*), and disperse frontal cortex neurons (*c*). No specific signal was seen after hybridization with the control probe NGF/118^{-} as shown for the periglomerular cells (*d*). Exposure time was 7 wk. Bars, (*a*) 1 mm; (*b–d*) 100 μm.

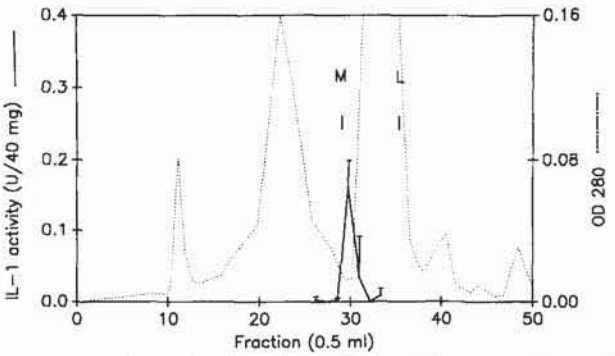

Figure 8. Column-fractionation of IL 1 activity. Frontal cortex homogenate (of 40 mg wet wt) has been applied on a Superose 12 column. Fractions that eluted between 14 and 17 kD have been measured (see Materials and Methods) for their IL 1 activity. The absorbance at 280 nm was continuously monitored (*dotted line*). All the IL 1 activity eluted as a single peak at 16 kD (*straight line*). Molecular weight markers: myoglobin (*M*; 17 kD) and lysozyme (*L*; 14.7 kD).

sized within the same cells. Labeled neurons were also observed in the cerebral cortex, restricted over neurons in the frontal cortex, however, complete identification of these cells was not possible (Fig. 7 *c*). In addition we could now detect cell bodies hybridizing with the NGF/118$^+$ DNA probe over the periglomerular cells of the olfactory bulb (Fig. 7 *b*), whereas no signal above background was seen in sections hybridized with the control probe NGF/118$^-$. (Fig. 7 *d*). As seen for the IL 1 mRNA, the specific labeling over periglomerular cells varied from strongly positive neurons to negative ones, presumably reflecting differential NGF synthesis by individual neurons.

IL 1 Activity

The IL 1 assay we used was a costimulator assay measuring IL 2 receptor expression induced on EL4 cells by IL 1 and a suboptimal dose of a phorbolester (LeMoal et al., 1988). Preliminary experiments showed that rat blood contains a phorbol ester independent activity (constituent of 30–40 kD), which drastically interfered with the bioassay used. We therefore decided to perfuse the animals and to fractionate the tissue samples by FPLC to separate other IL 2 receptor inducing activities as described by Tagaya et al. (1988). The peak activity found in various brain regions eluted slightly later than myoglobin (17 kD) and before lysozyme (14 kD) at a position corresponding to ~16 kD (Fig. 8). For comparison >80% of the IL 1 activity of the conditioned medium from activated rat macrophage cultures eluted at the same position (data not shown). Since macrophages release a variety of factors that could possibly interfere with our assay system we take this finding as a proof for the specificity of the method. Determination of the IL 1 activity in various brain regions after fractionation closely resembled the distribution pattern found by in situ hybridization. Although the values of corresponding tissue samples varied between different animals, they reflect a regionally consistent distribution of the IL 1 activity in all examined rat brains. The highest activity levels were found in the olfactory bulb, hippocampus, cerebellum, and the frontal cortex, reaching maximal levels of 10^{-3} U/mg wet weight (Table I). In comparison, IL 1 activity found in conditioned medium from activated rat macrophages (per 10^6 cells) was ~500–1,000 U. A 1,000-fold lower, but consistent level was observed in the striatum (Table I), where in situ hybridizations have indicated that probably glial cells are the IL 1β-specific source. The specificity of these values was further proven by the fact that rat specific polyclonal antibodies raised against IL 1 could adsorb up to 93% of the IL 1 activity found in frontal cortex or cerebellum (Table I).

Discussion

The only information on a possible involvement of IL 1β in the regulation of NGF synthesis in the CNS is based on the augmentation of NGF mRNA in the hippocampus after intraventricular injections of IL 1β (Spranger et al., 1990). However, these experiments did not provide evidence whether IL 1β also plays a role in the regulation of NGF synthesis under physiological conditions. We have now identified the cell types and areas in the adult rat brain that synthesize IL 1β mRNA as well as NGF mRNA, and found a regional colocalization of both mRNAs. NGF was shown to be regionally distributed in the mammalian brain, with the highest levels in areas innervated by magnocellular neurons present in the basal forebrain (Korsching et al., 1985; Shelton and Reichardt, 1986; Whittemore et al., 1986). Due to the limiting amounts of NGF mRNA a precise cellular localization has been successfully demonstrated only over the pyramidal cell layer of the hippocampus, granule cells of the dentate gyrus and over neurons found in the neocortex (Rennert and Heinrich, 1986; Ayer-Le Lièvre et al., 1988; Whittemore et al., 1988). These results are consistent with RNA blot analysis showing that hippocampus and neocortex have the highest NGF mRNA levels (see Korsching, 1987). The olfactory bulb is another area known to express NGF mRNA in relatively high quantities (see Korsching, 1987). In this paper we have shown by in situ hybridization that the periglomerular cells are the specific source of NGF mRNA in this region. Interestingly, periglomerular cells are interneurons lying in the glomerular layer which receives cholinergic input (Halasz and Sheperd, 1983), which demonstrates again that the target cells synthesize NGF. Of particular in-

Table I. IL-I Activity in Different Brain Regions of Three Individual Animals

Region	IL 1 activity				
	Animal	Animal	Animal	Average	SD
	10^{-3} U/mg wet wt				
Olfactory	1.25	1.5	0.1	0.95	0.75
Frontal cortex*	0.25	0.5	1.25	0.67	0.52
Hippocampus	1.0	0.5	0.5	0.67	0.29
Cerebellum*	0.5	0.13	0.5	0.37	0.21
Striatum	0.0	0.0025	0.0005	0.0025	0.0025

* One frontal cortex and one cerebellum were analyzed by the immunoadsorbtion protocol given in Materials and Methods. Compared with the control columns 93% (cerebellum) and 90% (frontal cortex) of the IL 1 activity were adsorbed.

Tissues have been fractionated by gel filtration and IL-1 activity was analyzed as described in Materials and Methods.

terest was the observation that the regional distribution found for NGF coincides with the determination of IL 1 activity. In addition, in situ hybridization demonstrated that IL 1β is predominantly expressed by neurons, which were shown to be positive for NGF. However, IL 1β was also present in other brain regions such as the hypothalamus, striatum, and septum, where the levels of NGF mRNA are at best at the limit of detectability (Whittemore et al., 1986), suggesting that IL-1β must have a different or additional function in these tissues (Breder et al., 1988). It is of interest that except for the hypothalamus glial cells are most likely responsible for the expression of IL 1β mRNA in these brain tissues. However, it was not possible to unequivocally identify whether astrocytes and/or microglial cells contain the IL 1β mRNA. Both cell types have been shown to synthesize IL-1 in vitro (Fontana et al., 1982; Giulian et al., 1986) but there is no evidence for a production under physiological conditions. Although we could demonstrate a partial colocalization of IL 1β and NGF, suggesting that IL 1β might be involved in the regulation of NGF synthesis also in the CNS, the physiological relevance of the observed results are hampered by the fact that hydropathicity profiles of IL 1β precursor apparently lack any regions that have sufficient hydrophobicity and length to qualify as a signal peptide (Lomedico et al., 1984). Whether IL 1 can be secreted despite these apparent shortcomings remains an open question. Recent reports have shown that only monocytes secrete IL 1 in both the precursor and the mature form (Hazuda et al., 1988), whereas transfected fibroblast cell lines were not able to release any of the two forms, despite a high level expression of the IL 1β precursor within the cells (Young et al., 1988). It is therefore likely that the used fibroblast cell lines lack some cellular factor(s) that allow the secretion and release of the intracellular IL 1β. To evaluate the function of IL 1 in the regulation of NGF synthesis under physiological conditions in the CNS it remains to be established whether neurons use a release mechanism similar to the one described for monocytes and how neuronal IL 1 synthesis and release is regulated.

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