Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 10352–10356, November 1991
Neurobiology

Expanded distribution of mRNA for nerve growth factor, brain-derived neurotrophic factor, and neurotrophin 3 in the rat brain after colchicine treatment

(neurotrophic factor/regulation/localization/*in situ* hybridization)

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Contributed by T. Hökfelt, August 16, 1991

ABSTRACT **The effect of intracerebroventricular injection of the mitosis inhibitor colchicine on expression of mRNA for nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin 3 was studied in the rat brain with *in situ* hybridization. Colchicine up-regulates mRNA for NGF and BDNF in many of the neuronal systems normally expressing these factors. In addition, after colchicine treatment NGF and BDNF mRNAs were localized in several brain areas where they normally cannot be detected. Thus, NGF mRNA was present, for example, in many motor nuclei and in the basal forebrain, and BDNF mRNA was seen in many nuclei in the brain stem and in catecholamine neurons, including dopamine neurons in the substantia nigra. The latter neurons have recently been shown to be sensitive to BDNF, and the present results show that these neurons can produce this factor themselves. A decrease in mRNA for BDNF and neurotrophin 3 was seen only in the granular-cell layer of the hippocampal formation. A strong hybridization signal for BDNF and neurotrophin 3 mRNA was also observed over several myelinated tracts in treated rats, supporting the hypothesis that glial cells as well as neurons can produce these trophic factors.**

The antimitotic agent colchicine is known to inhibit axonal transport of neuroactive compounds (1, 2) and to cause accumulation of transmitter-storage vesicles in cell somata (3). Subsequently colchicine has frequently been used in immunohistochemical studies to increase levels of transmitters, peptides, and related substances in cell bodies (4, 5). Colchicine arrests axonal transport due to microtubule disruption (6). However, it has been assumed that protein/peptide synthesis remains unaffected, leading to accumulation of various compounds in the cell bodies.

More recently colchicine has been shown to have a more complex action on neurons. For example, colchicine increases c-Fos levels in many brain areas (7) and causes changes in mRNA levels for a number of peptides, enzymes, and other substances (8). For instance, in the cholinergic basal forebrain system mRNA levels for choline acetyltransferase (acetyl-CoA:choline *O*-acetyltransferase, EC 2.3.1.6) are lowered, whereas mRNA levels for the peptide galanin are dramatically increased in this and many other systems (8). A similar up-regulation of galanin mRNA levels in basal forebrain neurons occurs after an electrolytic lesion of the hippocampus (9). In other systems it has been proposed that proteins up-regulated after lesions may be needed for restitution and survival and that transmitter-related compounds may be down-regulated (10). For example, the low-affinity 75-kDa nerve growth factor (NGF) receptor is up-regulated in motoneurons after axotomy (11) and after intracerebroventricular injection of colchicine (12).

During the last years much interest has been focused on NGF (13), the first member of a family of structurally related neurotrophic factors including brain-derived neurotrophic factor (BDNF) (14, 15), neurotrophin 3 (NT3; also called hippocampus-derived neurotrophic factor) (16–20), and neurotrophin 4 (21). NGF, BDNF, and NT3 are all expressed in the brain (16, 20, 22–27). Expression of NGF and BDNF in the brain appears to be regulated by neuronal activity (28–30), and the levels of mRNAs for both factors show a marked but transient increase during kindling epileptogenesis (31) as well as after cerebral ischemia and insulin-induced hypoglycemia (32).

The present study was undertaken to analyze to what extent intraventricular colchicine treatment may affect expression of mRNAs for NGF, BDNF, and NT3. Our results show that under these circumstances many more neurons in the brain express these factors at detectable levels as compared to control brains.

MATERIALS AND METHODS

Experimental Animals and Preparation of Tissue. Male Sprague–Dawley rats (body weight 150–180 g; Alab, Stockholm; Instituto de Neurobiología, Buenos Aires) (n = 24) were anesthetized with chloral hydrate (350 mg/kg), stereotaxically injected with 120 μg of colchicine (Sigma) in 20 μl of 0.9% NaCl into the lateral ventricle. Sham-operated controls received 20 μl of 0.9% NaCl. After 24-, 40-, or 48-hr survival, treated and sham-injected animals were sacrificed by decapitation, the brains were removed, dissected, frozen on microtome chucks, cut at 14 μm in a cryostat, and thaw-mounted onto ProbeOn microscope object slides.

***In Situ* Hybridization.** The tissues were hybridized according to published procedures (33). Synthetic oligonucleotide probes complementary to rat NGF mRNA (nucleotides 869–919) (34), rat BDNF mRNA (nucleotides 650–699) (23), and rat NT3/hippocampus-derived neurotrophic factor mRNA (nucleotides 667–717) (20) were used. The oligonucleotide probes were labeled at their 3′ end with adenosine 5′-[α-[^{35}S]thio]triphosphate (NEN) using terminal deoxynucleotidyltransferase (IBI) to a specific activity of ≈1 × 10^9 cpm/μg. After fixation in 10% (vol/vol) formalin, hybridization was done at 42°C for 15 hr in a formamide solution containing dithiothreitol, with the respective probe at 10^7 cpm/ml. After being rinsed, the sections were exposed to Amersham Hyperfilm-βmax film. Films were developed and fixed, the

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Abbreviations: NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; NT3, neurotrophin 3.

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sections were then dipped in Kodak NTB-3 photo emulsion, developed, fixed, counterstained with cresyl violet, and examined in a Nikon Microphot-FX microscope equipped with dark- and bright-field condensors. Film autoradiograms were quantified by microdensitometry (33). Four animals per group and five sections per animal were analyzed. The results are expressed as percentage value versus the control group.

RESULTS

In the following we describe the distribution of mRNA for NGF, BDNF, and NT3 in the rat brain, with emphasis on the localization after colchicine treatment. In this preliminary report we will mainly focus on distribution patterns that differ distinctly or exhibit a much stronger expression as compared with control rats. In no cases were *all* cells in any of these nuclei labeled.

NGF. In *control rats* NGF mRNA expression was observed in the hippocampal formation, with strongly labeled cells in hilus of the dentate gyrus (Fig. 1 *a* and *c*) and scattered positive cells in other hippocampal layers. After *colchicine treatment*, an increase was seen in the number and intensity of labeling in the polymorph layer of the dentate gyrus as well as in other hippocampal cell layers, particularly in the CA3 region (Fig. 1 *b* and *d*). Hybridization could now be seen in many other telencephalic areas, including the piriform (Fig. 2*e*), entorhinal, and perirhinal cortices with a particularly strong signal in the cingulate cortex. Also the frontal cortex, tenia tecta, and amygdaloid cortex contained NGF mRNA-positive cells, as well as numerous large neurons in the ventral forebrain (diagonal band nucleus, ventral pallidum) (Fig. 2*k*). At the *diencephalic level* positive cell groups were observed in the mediodorsal thalamic nucleus, the supramammillary complex (Fig. 2*g*), and the mammillary nucleus. In the *lower brain stem* many motor and some sensory nuclei expressed NGF mRNA, including the motor nucleus of the trigeminal nerve, the trochlear, facial, hypoglossal, dorsal vagal motor nuclei, and upper spinal motor neurons. Other

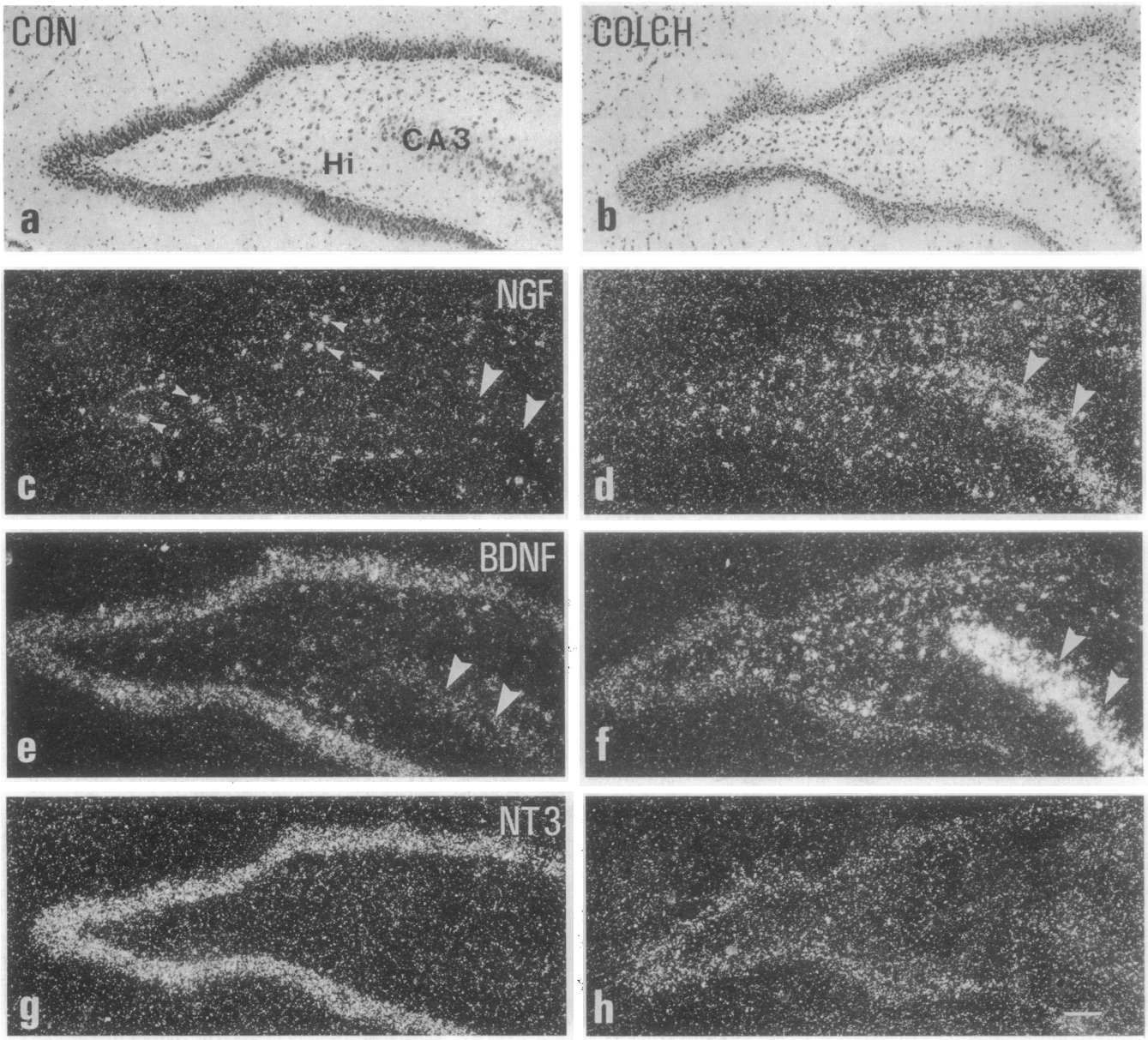

FIG. 1. Bright-field (*a* and *b*) and dark-field (*c–h*) photomicrographs of neurons in the dentate gyrus of control (CON) (*a*, *c*, *e*, and *g*) and colchicine (COLCH) (*b*, *d*, *f*, and *h*)-treated rats. (*a* and *b*) Nissl-stained sections of autoradiographs shown in *c* and *d*, where probes for NGF (*c* and *d*), BDNF (*e* and *f*), and NT3 (*g* and *h*) mRNAs were used. Large arrowheads point to CA3; small arrowheads show cells in hilus (Hi) region. (Bar = 100 μm.)

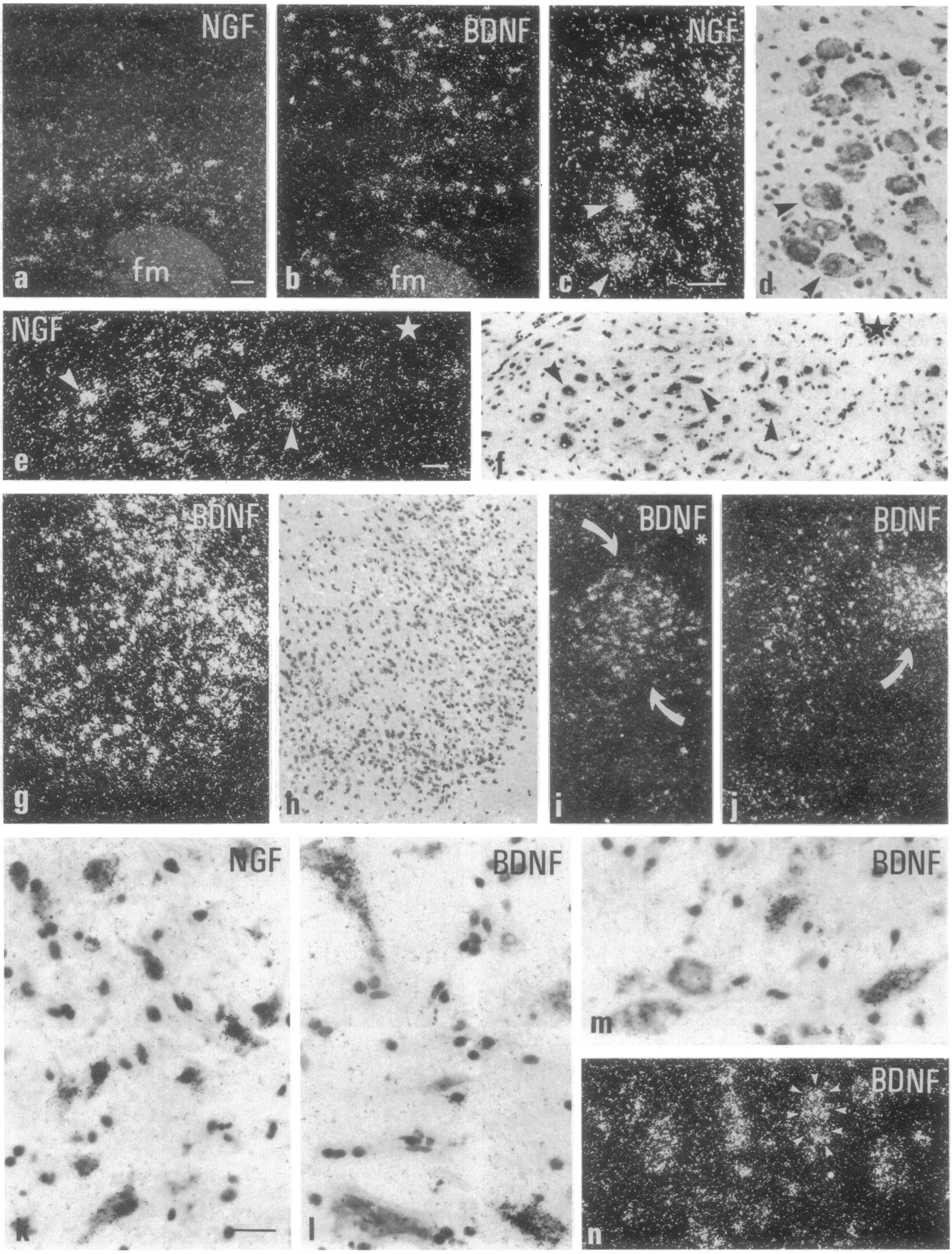

FIG. 2. Dark-field (*a–c, e, g, i, j,* and *n*) and bright-field (*d, f, h,* and *k–m*) photomicrographs from colchicine-treated rats hybridized with NGF (*a, c, e,* and *k*) or BDNF (*b, g, i, j,* and *l–n*) probes in the supramammillary region of the hypothalamus (*a* and *b*); mesencephalic nucleus of the trigeminal nerve (*c* and *d*); hypoglossal nucleus (*e* and *f*); piriform cortex (*g* and *h*); locus coeruleus (between curved arrows) (*i*); basal

positive nuclei were the mesencephalic trigeminal nucleus (Fig. 2*c*), the pontine reticular and reticulotegmental nuclei, the superior and inferior olive, large neurons in the medullary reticular formation, the nucleus raphé magnus, the gigantocellularis reticular nucleus including its pars alpha, the lateral reticular nucleus, and the solitary tract nucleus.

BDNF. In the *telencephalon* BDNF mRNA-expressing cell bodies were seen in many areas of *control rats*. In the hippocampal formation a distinct signal was seen in the CA1, CA2, and CA3 regions, the granular cell layer, as well as in single cells in the hilar region (Fig. 1*e*). Positive cells were found in neocortical regions, in the prefrontal cortex, the amygdaloid cortex, tenia tecta, and the piriform cortex. Also the anterior olfactory nucleus and the amygdaloid complex showed a distinct hybridization. At the *diencephalic level* positive cells were found in the medial tuberal nucleus, supramammillary region, and in the mediolateral thalamic nucleus. In the *lower brain stem* positive cells were observed in the locus coeruleus, the ventrolateral medulla oblongata, and in the inferior olive complex. After *colchicine treatment* a marked increase was observed in many areas containing BDNF mRNA in control rats, and many new positive cell groups were encountered. At the *telencephalic level* there was a marked up-regulation in the CA1, CA2, and CA3 in the hippocampus, and the number and intensity of the labeling of the hilar cells markedly increased. However, the number of grains overlying the granular cell layer decreased (Fig. 1*f*). The quantitative evaluation revealed a >3-fold increase in the CA3 region (Fig. 3). Areas with strong up-regulation were the piriform (Fig. 2*g*), deep perirhinal, cingulate, frontal, and parietal cortex (layers 2, 3, and 6), and the supramammillary region (Fig. 2*b*). New cell groups were observed in the amygdalohippocampal area and in the basal forebrain (diagonal band nucleus; few cells) and in *diencephalic areas* in many hypothalamic and thalamic nuclei (paraventricular nucleus, ventromedial nucleus, posterior hypothalamic area, parafascicular nucleus, midline thalamic areas, paraventricular thalamic, and the subthalamic nuclei). At the *mesencephalic level* positive cells were seen in the substantia nigra, and in the *lower brain stem* positive cells were seen in the mesencephalic sensory trigeminal nucleus, the pontine nuclei, the pontine reticular formation, the periaqueductal central grey, including the dorsal raphé nucleus, the dorsal and ventral parabrachial nuclei, the raphé magnus nucleus, the gigantocellular reticular nucleus, including its pars alpha, the solitary tract nucleus (Fig. 2*j*), the medullary reticular formation, the lateral reticular nucleus, the gracile nucleus, the external cuneate nucleus, inferior olive complex, and in a few neurons in several motoneuron groups (facial nucleus, ambiguus nucleus, motoneurons in upper cervical cord) as well as in cells in the region of catecholamine groups A1, A2, A5, and A6 (locus coeruleus), C1, and C2/C3. Finally, higher than background signal was observed over many myelinated tracts including the lateral olfactory tract, the myelinated bundles traversing the striatum, corpus callosum, and the pyramidal tracts.

NT3. NT3 mRNA was essentially confined to the hippocampal formation and cerebellum in control animals with a strong signal over the granular layer of the cerebellum (paraflocular lobe) and of the dentate gyrus (Fig. 1*g*), presumably part of CA2 and medial CA1. After colchicine treatment there was a strong reduction in labeling over the hippocampal granular layer (Fig. 1*h*). A distinct hybridization was seen over many myelinated tracts such as optic nerves, corpus callosum, and myelinated bundles in the caudate nucleus.

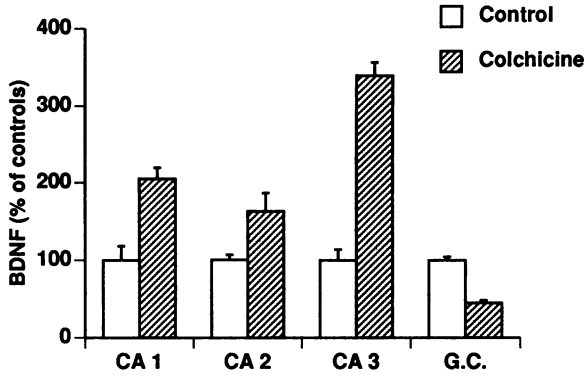

FIG. 3. Analysis of colchicine effect on BDNF mRNA in pyramidal cell layers CA1, CA2, CA3, and granular-cell layer (G.C.) of the dentate gyrus in the hippocampal formation. Data are expressed as percentage of controls (mean ± SEM).

DISCUSSION

The present results confirm several earlier studies showing marked differences between the number of neurons in the brain of untreated rats expressing the mRNA for NGF, BDNF, and NT3, whereby BDNF exhibits the most extensive distribution patterns (20–26, 35, 36), and NT3 has a very limited occurrence (20, 24, 25, 35). We here demonstrate a somewhat more detailed study on BDNF mRNA in the lower brainstem of untreated rats than in previous reports. The main finding of the present paper is, however, that treatment with colchicine at a high dose markedly up-regulates mRNA levels for both NGF and BDNF. Lower mRNA levels than in control rats were only seen in the granular-cell layer in the hippocampus, where mRNAs for both NT3 and BDNF markedly decreased, perhaps related to colchicine-induced degeneration of the granule cells (37).

NGF mRNA could be demonstrated in many more regions after colchicine treatment than in control rats. These regions included several cortical areas (piriform, entorhinal, perirhinal, amygdaloid cortices), the supramammillary region, and some other thalamic nuclei, numerous nuclei in the lower brainstem, including motor nuclei as well as raphé and reticular gigantocellular neurons, and the mesencephalic sensory trigeminal nucleus. Although BDNF mRNA was more extensively expressed in control rats than were NGF and NT3 mRNAs, as reported earlier (24–26), we also saw a marked increase in number of cells and intensity of labeling after colchicine treatment with >3-fold increase in BDNF mRNA in the CA3 region of the hippocampus. Areas with BDNF-positive cells included cortical, subcortical, and many brainstem nuclei. A particularly impressive up-regulation was seen in systems in the piriform cortex, supramammillary region, dorsal thalamic midline areas, and several other thalamic, hypothalamic, pontine, and medullary nuclei. Somewhat surprising was the strong hybridization signal over several myelinated tracts for both BDNF and NT3 mRNA after colchicine. Expression of NGF mRNA in central nervous system glia has recently been reported by Lu *et al.* (38). It is, therefore, possible that not only neurons, but in the case of BDNF and NT3, also glial elements express mRNA for

amygdaloid nucleus (curved arrow) (*j*); nucleus of the diagonal band (*k*); reticular gigantocellular nucleus (*l*); lateral reticular nucleus (*m*), and the striatum, where arrowheads surround a bundle of myelinated axons (*n*). The *c* and *d* pair, *e* and *f* pair as well as the *g* and *h* pair represent, respectively, the same sections under dark-field or bright-field illumination to show cellular labeling (arrows). fm, Fasciculus mammillothalamicus; ☆, central canal; *, fourth ventricle. [Bars = 50 μm (*a* = *b* = *g* = *h* = *i* = *j* = *n*), (*c* = *d*), (*e* = *f*), (*k* = *l* = *m*).]

growth factors under conditions of severe stress as a survival reaction.

BDNF mRNA can be detected transiently postnatally in several cortical regions and in brainstem areas including substantia nigra and the nucleus raphé magnus/gigantocellular reticular nuclei (39). Interestingly colchicine treatment of adult rats can induce a reexpression of BDNF mRNA in these regions. Further evidence for plasticity of BDNF and NGF mRNA expression in the brain has been obtained from studies showing that kindling induced by electrical stimulation in the hippocampus leads to a marked and transient increase in mRNA for NGF and BDNF in several cortical regions, whereas no effect was seen on levels of NT3 mRNA (31). Moreover, the levels of BDNF and NGF mRNAs show a transient increase in the dentate gyrus after cerebral ischemia and insulin-induced hypoglycemia (32). However, in contrast to expression after colchicine administration, these treatments appear to only increase NGF and BDNF mRNA in areas of the brain that express these factors in untreated animals.

BDNF mRNA was found in several noradrenaline (A6) and dopamine neurons of colchicine-treated rats. The localization of BDNF mRNA in dopamine neuron is interesting because recent studies have shown that BDNF supports the survival of dopaminergic neurons of the substantia nigra in culture (40, 41) and because BDNF mRNA is expressed transiently postnatally in these neurons (39). The present study also shows that in colchicine-treated rats large neurons in the basal forebrain express NGF mRNA. It is possible that these neurons belong to the cholinergic basal forebrain neurons (42), which are known to have NGF receptors (43, 44) and to take up and retrogradely transport NGF (45, 46). In fact, extensive studies have shown that NGF is a trophic factor for these neurons (47). In the adult brain NGF has so far been shown to be synthesized only in the target areas of these neurons, such as the hippocampus (34, 48, 49), whereas NGF mRNA has been detected by Northern (RNA) blot in embryonic basal forebrain tissue (50). The present results suggest that these neurons after colchicine treatment can produce this factor themselves, opening up the possibility of an autocrine/paracrine mode of trophic stimulation by NGF in the basal forebrain after colchicine treatment, as may be so also for BDNF and catecholamine neurons. In agreement with this hypothesis Pioro and Cuello (12) showed with immunohistochemistry increased levels of the low-affinity NGF receptor after colchicine treatment, for example, in many motor nuclei in the brainstem, such as the facial and hypoglossal nuclei—that is, nuclei that express NGF mRNA after colchicine treatment. Taken together, the possibility therefore exists that a trophic factor does not necessarily have to be produced in the target areas of sensitive neurons and then be retrogradely transported to the cell body but that the trophic factor could act directly on the cell in which it is produced. This mechanism would provide a fast and efficient way to obtain growth and survival-promoting effects.

This work has been supported by the National Institute of Mental Health (MH 43230-02), the Swedish Medical Research Council (04x-2887), Konung Gustav V:s och Drottning Victorias Stiftelse, Fredrik och Ingrid Thurings Stiftelse and Marianne och Marcus Wallenbergs Stiftelse, United States Grants AG04418 and NS09199 and Fundación Antorchas. M.J.V. was supported by a grant from the Wenner-Gren Foundation.

1. Dahlström, A. (1968) *Eur. J. Pharmacol.* **5**, 111–113.
2. Kreutzberg, G. W. (1969) *Proc. Natl. Acad. Sci. USA* **62**, 722–728.
3. Hökfelt, T. & Dahlström, A. (1971) *Z. Zellforsch. Mikrosk. Anat.* **119**, 460–482.
4. Barry, J., Dubois, M. P. & Poulain, P. (1973) *Z. Zellforsch. Mikrosk. Anat.* **146**, 351–366.
5. Hökfelt, T., Ljungdahl, A., Terenius, L., Elde, R. & Nilsson, G. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3081–3085.
6. Hanson, M. & Edström, A. (1978) *Int. Rev. Cytol.*, Suppl. 7, 373–402.
7. Ceccatelli, S., Villar, M. J., Goldstein, M. & Hökfelt, T. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 9569–9573.
8. Cortés, R., Ceccatelli, S., Schalling, M. & Hökfelt, T. (1990) *Synapse* **6**, 369–391.
9. Cortés, R., Villar, M. J., Verhofstad, A. & Hökfelt, T. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 7742–7746.
10. Lieberman, A. R. (1971) *Int. Rev. Neurobiol.* **14**, 49–124.
11. Ernfors, P., Hallböök, F., Ebendal, T., Shooter, E. M., Radeke, M. J., Misko, T. P. & Persson, H. (1989) *Neuron* **2**, 1605–1613.
12. Pioro, E. P. & Cuello, A. C. (1990) *Neuroscience* **34**, 57–87.
13. Levi-Montalcini, R. (1987) *Science* **237**, 1154–1162.
14. Barde, Y.-A., Edgar, D. & Thoenen, H. (1982) *EMBO J.* **1**, 549–553.
15. Leibrock, J., Lottspeich, F., Hohn, A., Hofer, M., Hengerer, B., Masiakowski, P., Thoenen, H. & Barde, Y. A. (1989) *Nature (London)* **341**, 149–152.
16. Hohn, A., Leibrock, J., Bailey, K. & Barde, Y. A. (1990) *Nature (London)* **344**, 339–341.
17. Maisopierre, P. C., Bellucio, L., Squinto, S., Ip, N. Y., Furth, M. E., Lindsay, R. M. & Yancopoulus, G. D. (1990) *Science* **247**, 1446–1451.
18. Rosenthal, A. V. G. D., Nguyen, T., Lewis, M., Shih, A., Laramee, G. R., Nikolics, K. & Winslow, J. W. (1990) *Neuron* **4**, 767–773.
19. Kaisho, Y., Yoshimura, K. & Nakahama, K. (1990) *FEBS Lett.* **266**, 187–191.
20. Ernfors, P., Ibáñez, C. F., Ebendal, T., Olson, L. & Persson, H. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 5454–5458.
21. Hallböök, F., Ibáñez, C. F. & Persson, H. (1991) *Neuron* **6**, 845–858.
22. Ayer-LeLievre, C., Olson, L., Ebendal, T., Seiger, Å. & Persson, H. (1988) *Science* **240**, 1339–1341.
23. Hofer, M., Pagliusi, S. R., Hohn, A., Leibrock, J. & Barde, Y.-A. (1990) *EMBO J.* **9**, 2459–2464.
24. Ernfors, P., Wetmore, C., Olson, L. & Persson, H. (1990) *Neuron* **5**, 511–526.
25. Phillips, H. S., Hains, J. M., Laramee, G. R., Rosenthal, A. & Winslow, J. W. (1990) *Science* **250**, 290–294.
26. Wetmore, C., Ernfors, P., Persson, H. & Olson, L. (1990) *Exp. Neurol.* **109**, 141–152.
27. Lauterborn, J. L., Isackson, P. J. & Gall, C. M. (1991) *J. Comp. Neurol.* **306**, 439–446.
28. Zafra, F., Hengerer, B., Leibrock, J., Thoenen, H. & Lindblom, D. (1990) *EMBO J.* **9**, 3545–3550.
29. Gall, C., Murray, K. & Isackson, P. J. (1991) *Mol. Brain Res.* **9**, 113–123.
30. Ballarín, M., Ernfors, P., Lindefors, N. & Persson, H. (1991) *Exp. Neurol.*, in press.
31. Ernfors, P., Bengzon, J., Kokaia, Z., Persson, H. & Lindvall, O. (1991) *Neuron* **7**, 165–176.
32. Lindvall, O., Ernfors, P., Bengzon, J., Kokaia, Z., Smith, M.-L., Siesjö, B. K. & Persson, H. (1992) *Proc. Natl. Acad. Sci. USA*, in press.
33. Schalling, M., Franco-Cereceda, A., Hemsen, A., Dagerlind, Å., Seroogy, K., Persson, H., Hökfelt, T. & Lundberg, J. M. (1991) *Neuroscience* **41**, 753–766.
34. Whittemore, S. R., Ebendal, T., Lärkfors, L., Olson, L., Seiger, Å., Strömberg, I. & Persson, H. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 817–821.
35. Jones, K. R. & Reichadt, L. F. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 8060–8064.
36. Isackson, P. J., Huntsman, M. M., Murray, K. D. & Gall, C. M. (1991) *Neuron* **6**, 937–948.
37. Goldschmidt, R. B. & Steward, O. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3047–3051.
38. Lu, B., Yokoyama, M., Dreyfus, C. F. & Black, I. B. (1991) *J. Neurosci.* **11**, 318–326.
39. Friedman, W. J., Olson, L. & Persson, H. (1991) *Eur. J. Neurosci.*, in press.
40. Hyman, C., Hofer, M., Barde, Y.-A., Juhasz, M., Yancopolous, G. D., Squinto, S. P. & Lindsay, R. M. (1991) *Nature (London)* **350**, 230–232.
41. Knüsel, B., Winslow, J. W., Rosenthal, A., Burton, L. E., Seid, D. P., Nikolics, K. & Hefti, F. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 961–965.
42. Wainer, B. H., Levey, A. I., Mufson, E. J. & Mesulam, M. M. (1984) *Neurochem. Int.* **6**, 163–182.
43. Richardson, P. M., Verge, V. M. K. & Riopelle, R. J. (1986) *J. Neurosci.* **6**, 2312–2321.
44. Riopelle, R. J., Richardson, P. M. & Verge, V. M. K. (1985) *Proc. Soc. Neurosci.* **11**, 1056.
45. Schwab, M. E., Otten, U., Agid, Y. & Thoenen, H. (1979) *Brain Res.* **168**, 473–483.
46. Seiler, M. & Schwab, M. E. (1984) *Brain Res.* **300**, 33–39.
47. Thoenen, H., Bandtlow, C. & Heumann, R. (1987) *Rev. Physiol. Biochem. Pharmacol.* **109**, 145–178.
48. Korsching, S., Auburger, G., Heumann, R., Scott, J. & Thoenen, H. (1985) *EMBO J.* **4**, 1389–1393.
49. Shelton, D. L. & Reichardt, L. F. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 2714–2718.
50. Lu, B., Buck, C. R., Dreyfus, C. F. & Black, I. B. (1989) *Exp. Neurol.* **104**, 191–199.