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Regulation of Neuropeptide Expression in the Brain by Neurotrophins

Potential Role In Vivo

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Abstract

Neurotrophins, which are structurally related to nerve growth factor, have been shown to promote survival of various neurons. Recently, we found a novel activity of a neurotrophin in the brain: Brain-derived neurotrophic factor (BDNF) enhances expression of various neuropeptides. The neuropeptide differentiation activity was then compared among neurotrophins both in vivo and in vitro. In cultured neocortical neurons, BDNF and neurotrophin-5 (NT-5) remarkably increased levels of neuropeptide Y and somatostatin, and neurotrophin-3 (NT-3) also increased these peptides but required higher concentrations. At elevating substance P, however, NT-3 was as potent as BDNF. In contrast, NGF had negligible or no effect. Neurotrophins administered into neonatal brain exhibited slightly different potencies for increasing these neuropeptides: The most marked increase in neuropeptide Y levels was obtained in the neocortex by NT-5, whereas in the striatum and hippocampus by BDNF, although all three neurotrophins increased somatostatin similarly in all the brain regions examined. Overall spatial patterns of the neuropeptide induction were similar among the neurotrophins. Neurons in adult rat brain can also react with the neurotrophins and alter neuropeptide expression in a slightly different fashion. Excitatory neuronal activity and hormones are known to change expression of neurotrophins. Therefore, neurotrophins, neuronal activity, and hormones influence each other and all regulate neurotransmitter/peptide expression in developing and mature brain. Physiological implication of the neurotransmitter/peptide differentiation activities is also discussed.

Index Entries: Nerve growth factor; brain-derived neurotrophic factor; neurotrophin, neuropeptide Y; somatostatin; substance P; neocortex; striatum; hippocampus; seizure; plasticity.

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Introduction

Each neuron is characterized by a particular set of cellular features, such as cellular morphology, transmitter phenotype, and projection pathway. The neuronal phenotypes are determined by not only imprinted cell fate but also their interaction with many diffusible intercellular signals provided by the surrounding environment. The phenotypic combinations of neurotransmitters found in individual neurons represent one of the most complex and diverse classes among many neuronal features. The number of neurotransmitters, including neuropeptides, thus far identified totals more than one hundred. However, each individual neuron chooses an appropriate set of neurotransmitters and neuropeptides during development, and releases these transmitters into synapses to pass correct signals to target cells. Even in adulthood, mature neurons can change dynamically the rate of synthesis and release of the neurotransmitters and neuropeptides in response to various types of stimuli. It has been suggested that regulated synthesis and release of neurotransmitters contribute to synaptic plasticity and maintain homeostasis of the nervous system. Therefore, many neurological and psychological disorders may often be associated with abnormal expression of neurotransmitters and neuropeptides.

What kinds of signals can alter the expression of neurotransmitters and neuropeptides? Previous studies on peripheral neurons have suggested that production of neurotransmitters and neuropeptides can be influenced by diffusible protein factors that are synthesized by many cell types, including glial cells, target cells and hematopoietic cells (Patterson and Nawa, 1993). For example, cholinergic differentiation factor (leukemia inhibitory factor or LIF) and ciliary neurotrophic factor (CNTF) similarly alter neurotransmitter/peptide phenotypes in sympathetic neurons (Fukada, 1985; Ernsberger et al., 1989; Saadat et al., 1989; Yamamori et al., 1989; Nawa et al., 1990; Rao et al., 1992). Nerve growth factor (NGF) controls the expression of substance P (SP) and calcitonin gene-related peptide (CGRP) in sensory neurons (Lindsay and Harmar, 1989). Ciliary parasympathetic neurons initiate somatostatin (SOM) expression in response to activin (Coulombe et al., 1992). These activities can be observed not only during normal development of the peripheral nervous system (PNS), but are also induced in response to nerve injury and inflammation (Jonakait and Schotland, 1990; Freidin and Kessler, 1991; Hyatt-Sachs et al., 1993). Such observations in the PNS suggested the possibility that diffusible protein factors could also control the expression of neurotransmitters and neuropeptides in the central nervous system (CNS).

Many pharmacological and physiological studies have demonstrated that neuropeptide expression in the CNS can be affected by drugs (Bannon et al., 1987; Tempel et al., 1990; Gerfen et al., 1991; Wahlestedt et al., 1991), steroid hormones (Simerly and Swanson, 1987; Baldino et al., 1988; Simerly et al., 1989; Kasper et al., 1992), and neuronal activity (Hendry et al., 1988; Agoston et al., 1991; Lindefors et al., 1991; Tolon et al., 1994). Since diffusible protein factors are known to mediate such alterations in neuropeptide expression in the PNS (Fukada, 1980; Rao et al., 1992; Shaiack et al., 1992), the effects of drugs, hormones, or electrical activity observed in the CNS could be, in part, also mediated by such protein factors. However, the regulation of neuropeptide expression by protein factors has not been elucidated well in the CNS.

Recently, we have found that neurotrophins have strong peptidergic differentiation activities in the CNS both in vivo and in vitro. In this paper, we present our new results as well as summarize the previous ones, and discuss potential physiological significance of the activities.

Differentiation vs Trophic Factors

Diffusible protein factors acting on neurons were classified into several groups according to their biological activities; growth factor, survival factor, trophic factor, differentiation fac-

tor, and so on. At present, however, such a distinction has become ambiguous because many neuronal factors can often exert multiple activities. Good examples are NGF and cholinergic differentiation factor (CDF). NGF was initially identified by its activity supporting survival of peripheral neurons (Levi-Montalcini, 1987) and recently shown to enhance neuronal differentiation of adrenal pheochromocytoma (Greene and Tischler, 1976) and Purkinje neurons (Cohen-Cory et al., 1991), axonal regeneration of sensory neurons (Lindsay, 1988), and to increase phenotypic expression of neurotransmitters and neuropeptides (Hefti et al., 1985; Martinez et al., 1985; Lindsay and Harmar, 1989; Smeyne et al., 1994). Conversely, CDF (Fukada, 1985; Yamamori et al., 1989), which was initially found to induce cholinergic phenotype in cultured sympathetic neurons, is now known to enhance differentiation of sensory neurons (Murphy et al., 1991) and support motoneuron survival in culture (Martinou et al., 1992). These two neuronal factors, NGF and CDF, form two distinct families: One of the families, the so-called neurotrophins, consists of NGF, BDNF (Barde et al., 1982; Leibrock et al., 1989), neurotrophin-3 (NT-3) (Hohn et al., 1990; Maisonpierre et al., 1990), neurotrophin-5 (NT-5) (Berkemeir et al., 1991) (identical to NT-4 [Halböök et al., 1991]), and neurotrophin-6 (NT-6) (Gotz et al., 1994) whereas CDF family includes CNTF, oncostatin-M (Bazan, 1991) and CDF/LIF itself. Among the CDF family, each member exerts similar but distinct biological activities on peripheral neurons (Rao et al., 1992; Fann and Patterson, 1993).

Neuronal factors have often been purified and identified by using culture supernatant from non-neuronal cells (conditioned medium; CM) (Nawa and Sah, 1990). Taking a similar approach, we attempted to identify novel peptidergic differentiation factor(s) acting on CNS neurons. A protein fraction from heart-cell-conditioned medium (CM) was added to neuronal cultures prepared from embryonic rat neocortices. Levels of neuropeptide expression were monitored by RNA blotting for mRNA for neuropeptide precursors (Fig. 1). The CM increased levels of mRNA for neuropeptide Y precursor (referred to NPY mRNA hereafter) twofold and levels of SOM mRNA by 30%, whereas it suppressed cholecystokinin mRNA (CCK mRNA). Glial contamination in culture was minimized to <3%. Thus, it is unlikely that the alteration in peptidergic mRNA levels reflected glial responses. In addition, the neuronal density and mRNA levels for GABA synthetic enzyme (glutamic acid decarboxylase; GAD) stayed constant, suggesting that the mRNA alteration did not result from enhanced survival of a particular neuronal population. Before engaging in the purification of the responsible protein factor(s), we examined whether any of the known neuronal factors might exhibit an identical activity. Using the same in vitro assay, we found that BDNF mimics this activity of increasing the expression of NPY and SOM.

Neuropeptide Differentiation Activities of Neurotrophins in Culture

BDNF elevated peptide levels of NPY and SOM in GABAergic neocortical neurons in culture, but its effect on CCK and GABA was much less significant (Nawa et al., 1993). mRNA levels for the neuropeptides were also similarly increased by the addition of BDNF, although it is unknown whether the mRNA increase was caused by an enhanced transcription rate or mRNA stability. However, these results suggest that BDNF enhances neuropeptide synthesis rather than inhibited neuropeptide release or degradation. BDNF is known to prevent glutamate-induced neuronal death (Lindholm et al., 1993). Thus, glutamate receptor blockers were supplemented to culture media in order to monitor only the differentiation activity of BDNF and not its neuronal survival promoting activity. Furthermore, since delaying the addition of BDNF to the culture medium did not influence the magnitude of neuropeptide induction, the peptidergic differ-

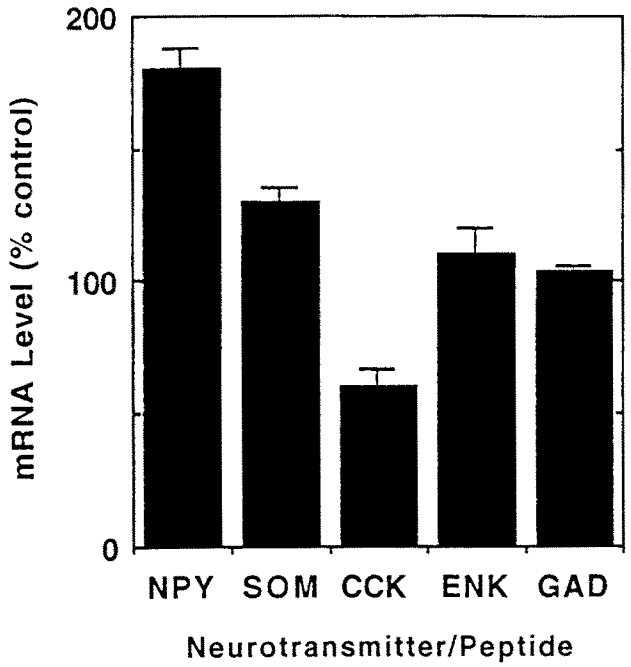
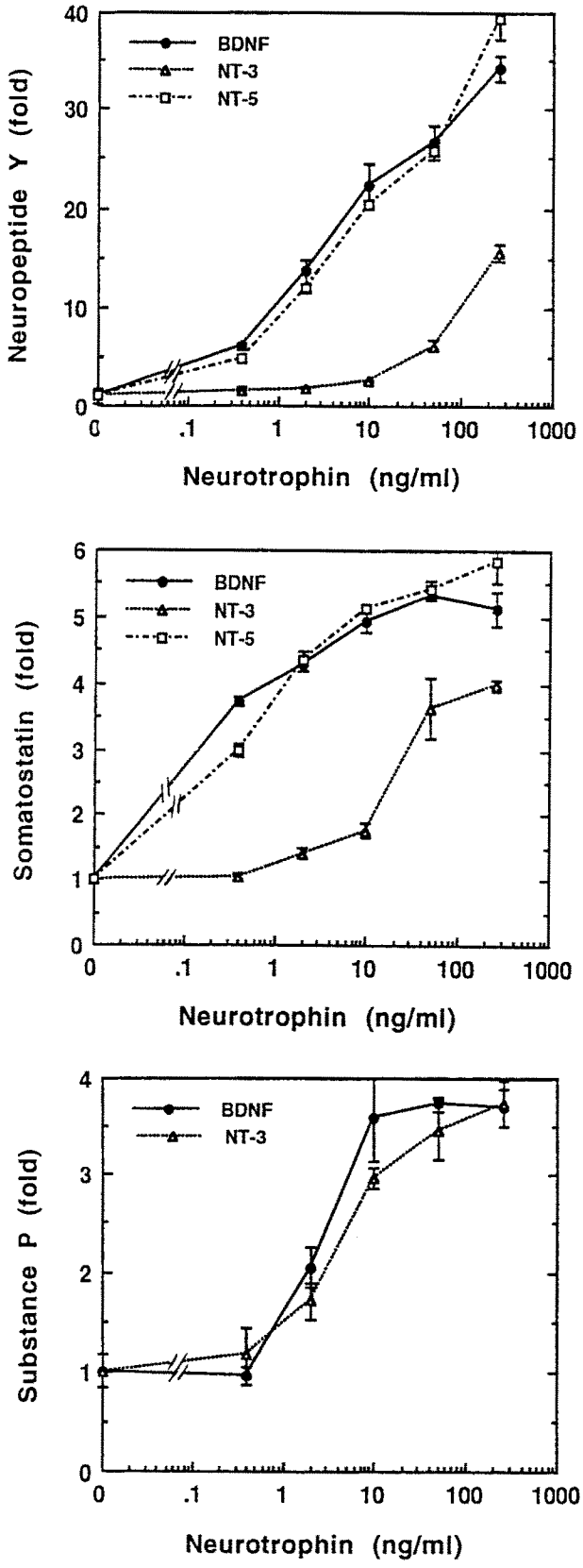


Fig. 1. Effects of heart-cell-conditioned media on neurotransmitter/peptide expression. The whole cerebral neocortices of embryonic rats (E18) were mechanically dissociated and plated onto laminin-coated dishes. Neurons were maintained in Dulbecco's modified Eagle's medium (DMEM) containing pure glutamine (2 m*M*), nutrient mixture N2 and 20 μ*M* AP-5 as described in our previous report (Nawa et al., 1993). One day after plating, the medium was supplemented with 20% (v/v) of heart-cell-conditioned media (Fukada, 1985). Cultures were treated with antimitotic reagent cytosine arabinoside (5 μ*M*) to suppress glial proliferation. This treatment reduced glial population to <3% as monitored by immunohistochemistry for glial fibrillary acidic protein. After 5 d, total RNA was extracted from cultures and mRNA levels for GAD and neuropeptides (i.e., neuropeptide Y, NPY; somatostatin, SOM; cholecystokinin, CCK; enkephalin, ENK) were analyzed by Northern blotting. The radioactivity in a positive band of each RNA was measured by a Fuji Bioimage Analyzer (BAS 2000) (n = 2).

entiation activity of BDNF is irrelevant to its survival-promoting activity presumably reported previously (Alderson et al., 1990; Hyman et al., 1991; Soppet et al., 1991; Ernfors et al., 1994).

The goal of the next set of experiments was to compare members of the neurotrophin family for their neuropeptide induction properties. Various concentrations of neurotrophins (i.e., NGF, BDNF, NT-3, and NT-5) were supplemented to neocortical cultures prepared from embryonic d 18 rats, and then, after 4 d, neuropeptide levels in cultured neurons were measured by radioimmunoassay (Fig. 2). BDNF increased contents of NPY and SOM in a dose-dependent manner. It is noteworthy that NPY content was increased more than 30-fold with the highest concentration of BDNF, suggesting neuropeptide expression of the cortical neurons is very plastic at this stage. NT-5 showed the same dose-response curves as BDNF. Only at higher concentrations was NT-3 able to elevate levels of the neuropeptides NPY and SOM. In contrast, both BDNF and NT-3 exhibited a similar dose-response curve for SP induction. However, NGF had little or no influence on the neuropeptides. Since NT-5 is a ligand for both BDNF receptors (*trkB* gene products) and NGF receptors (*trk* gene products) (Berkemeier et al., 1991), it is not surprising that NT-5 showed a dose-response curve similar to that of BDNF. In contrast, it is unclear whether the effect of NT-3 on SP reflected the stimulation of NT-3 receptors (*trkC* gene products) or its crossreactivity to *trkB* receptors (Soppet et al., 1991). Although a majority of neocortical neurons are known to express *trkB* receptor mRNA, only 8–10% of cultured neocortical neurons altered their neuropeptide expression in response to BDNF. Thus, it remains to be determined which types of neurotrophin receptors, including their truncated forms (Klein et al., 1990; Tsoulfas et al., 1993; Valuenzuela et al., 1993), are expressed by the cortical neurons and how they are involved in the peptidergic differentiation.

The effects of neurotrophins on other types of neurons were also characterized in cultures prepared from striatum and hippocampus (Mizuno et al., 1994). In striatal cultures, BDNF, but neither NGF nor NT-3 (20 ng/mL), increased NPY and SOM contents. The extent of this induction was similar to the one



observed on cultured neocortical neurons. However, effects of neurotrophins on GABAergic properties in striatal cultures were much more pronounced compared to those seen in neocortical cultures: BDNF elevated GABA levels threefold in striatal neurons. Subsequent biochemical analyses demonstrated that the increase of GABA levels resulted from a ninefold increase in holo-enzyme activity of GAD and a threefold increase in neuronal GABA uptake activity. From that respect, an influence of BDNF on GAD-immunoreactivity was most remarkable: BDNF increased the frequency of GAD-immunoreactive striatal neurons from 7 to 40%. A similar increase in GABA levels was observed in vivo in the striatum after intraventricular injection of BDNF (Mizuno et al., 1994). The GABAergic effect of neurotrophins is not limited to striatal neurons. Recently, Hyman et al. (1994) reported that neurotrophins can increase GAD activity as well as GABA uptake in cultured mesencephalic neurons. In contrast to the effects on cortical and striatal neurons, the neurotrophins exerted a modest influence on neuropeptides in cultured hippocampal neurons (H. N., unpublished data). This confirms previous reports that hippocampal neurons cannot alter GABAergic expression in response to neurotrophins (Collazo et al., 1992; Ip et al., 1993). Since the basal NPY levels were relatively high in hip-

Fig. 2. Dose-responsive increases of neuropeptides with neurotrophins. Neocortical cultures (Nawa et al., 1993) were treated with various concentrations of human recombinant neurotrophins; BDNF, NT-3, NT-5, and NGF. After 4 d in culture, peptides were extracted from the cultures with 50 m*M* acetic acid containing 1 m*M* EDTA. Peptide levels for neuropeptide Y, somatostatin, and substance P were measured by radioimmunoassay (Nawa and Sah, 1990). Each peptide increase was plotted as a ratio to a control value. There was no significant difference of neuronal densities among the cultures as described before (Nawa et al., 1993). Only the highest concentration of NGF (250 ng/mL) had very small effect on the neuropeptides; 1.72 ± 0.01-fold increase in NPY, 1.17 ± 0.04-fold increase in SOM, and 0.95 ± 0.09-fold increase in SP.

pocampal cultures, the overall effect of BDNF on NPY might be underestimated.

BDNF mRNA is expressed abundantly in most of the brain regions, with the highest concentrations in the hippocampus and neocortex (Maisonpierre et al., 1990). Expression of BDNF mRNA is initiated at late embryonic stages when peptidergic neurons are first observed (Friedman et al., 1991a). mRNA for BDNF receptors (*trkB* and *trkC*) is also known to be distributed in many brain regions (Ringstedt et al., 1993) including the neocortex, hippocampus, and striatum. In contrast, expression of NT-3 mRNA is fairly restricted to the hippocampus (Friedman et al., 1991b). NT-5 mRNA is present as early as embryonic d 13 in rat CNS and its expression is developmentally regulated after birth in various brain regions (Berkemeier et al., 1991; Timmusk et al., 1993). Therefore, it is expected that endogenous neurotrophins may exert a widespread spectrum of biological activities on a variety of neurons in mature and immature brain.

Neuropeptide Differentiation Activities of Neurotrophins In Vivo

To demonstrate that the neurotransmitter/peptide differentiation activities of neurotrophins do not reflect culture artifacts, we administered neurotrophins into the neonatal brain and examined their effects on neuropeptides. BDNF was injected twice into rat cerebroventricle during the first week postnatal, and immunoreactivity for NPY, SOM, SP, CCK, and enkephalin (ENK) was examined by radioimmunoassay and immunohistochemistry (Nawa et al., 1994). NPY and SP induction was observed in many brain regions centered around the BDNF injection site; neocortex, striatum, hippocampus, thalamus, and hypothalamus. Among the brain regions, the effect of BDNF on NPY and SP was most pronounced in the frontal neocortex where the cannula was inserted; an 11-fold increase of NPY and a 26-fold increase of SP levels in the ipsilateral hemisphere; a sevenfold increase of NPY and a 12-fold increase of SP levels in the contralateral hemisphere. In contrast, the effects of BDNF on SOM, CCK, and ENK were modest and limited to certain brain regions. The observation that exogenous BDNF acts on various types of CNS neurons in vivo is consistent with our results obtained in culture. The question about the role of *endogenous* BDNF in phenotypic differentiation of CNS neurons is now being examined. Consistent with our results, targeted disruption of the BDNF gene results in 35% reduction in the NPY-IR in the cortex and hippocampus (Jones et al., 1994). In the present study, we further analyzed quantitative and qualitative differences of the neurotrophins' effects in vivo.

Seven micrograms of BDNF, NGF, and NT-3 and 2.5 μg of NT-5 were injected into the lateral ventricle of neonatal rats (postnatal d 3) as described (Nawa et al., 1994). A higher concentration of NT-5 could not be used because of its poor solubility. Brains were dissected 43 h later, and levels of NPY, SOM, SP, CCK, and ENK were measured by radioimmunoassay in the anterior neocortex, striatum, and hippocampus (Fig. 3). Similar effects on NPY, SOM, and SP were found for BDNF, NT-3, and NT-5. NGF, as previously reported (Nawa et al., 1994), showed no significant neurotransmitter/peptide-inducing activity in any of the brain regions examined. A single injection of the lower amount of NT-5 seemed equally potent at inducing NPY expression (12-fold) as the higher dose of BDNF in the anterior neocortex. Only a fivefold increase in NPY levels was obtained when the equivalent lower dose of BDNF was injected in our in vivo dose-response study (Nawa et al., 1994). The same potency is also observed in the induction of SOM in the neocortex, striatum, and hippocampus. Since NT-5 can be a ligand for both *trkB* and *trk* receptors, and a ligand for the *trk* receptor, NGF, had no effect on the peptide levels, we can assume that the NT-5 effect in vivo is mediated largely through the *trkB* receptor. Therefore, the stronger peptidergic differentia-

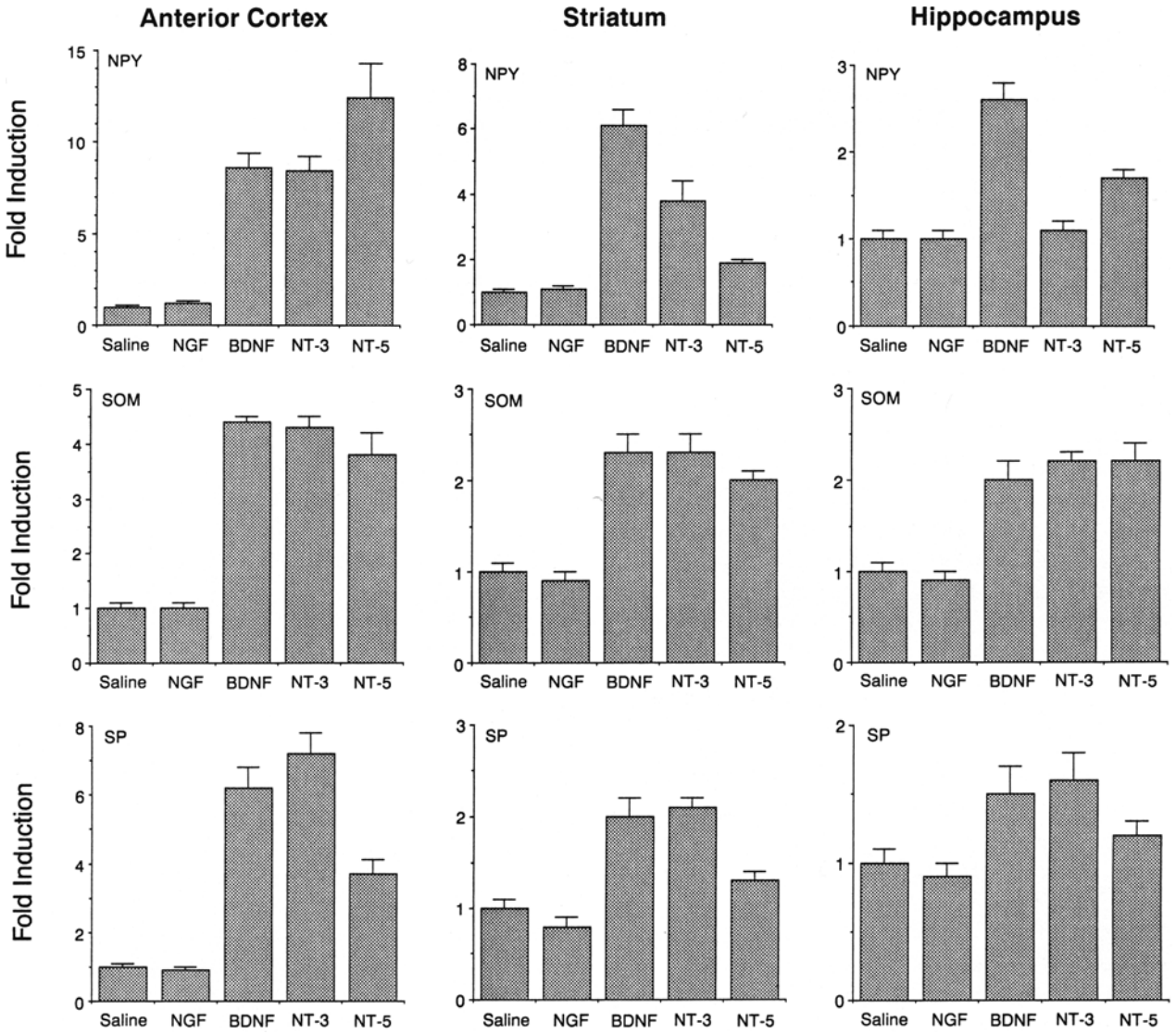


Fig. 3. Effects of neurotrophins in vivo on NPY, SOM, and SP levels. Seven micrograms of BDNF, NF, NT-3, and 2.5 μg of NT-5 were injected into the lateral ventricle of neonatal rats as described (Nawa et al., 1994). Brains were dissected 48 h later when peptide induction is maximal (Nawa et al., 1994), and NPY, SOM, and SP were measured by radioimmunoassay in the anterior neocortex, striatum, and hippocampus of each animal (n = 3–5). Results of the RIA are plotted as fold induction when compared to values in saline-injected animals.

tion activity of NT-5 in vivo might reflect a real property of the neurotrophin in the developing brain since NT-5 and *trkB* (Ernfors et al., 1992), but not BDNF (Timmusk et al., 1993), are present as early as embryonic d 13 in rat cortical neuroepithelium. Although the real NT-5 protein concentration in the neonatal brain is unknown, it is possible that there is some "priming" phenomenon or that enough endogenous NT-5 is present to add up to our injection of exogenous NT-5 and reaches the threshold necessary for this potent induction. Alternatively, clearance/degradation or pharmacological properties of NT-5 might be quite different from those of BDNF in vivo. A similar disparity between the concentrations of neurotrophins

necessary for the maximal activities was discussed in the previous section.

NT-3 also appears to be an important in vivo factor in the regulation of neurotransmitter/peptide expression. Like BDNF, it upregulated levels of NPY (eightfold), SOM (4.5-fold), and SP (eightfold) in the anterior neocortex. In contrast, CCK and leucine-Enk showed very little responsiveness to NT-3 and NT-5 as previously reported for BDNF (data not shown). This observation is consistent with the results in culture that NT-3 exhibited lower potency than BDNF in the dose-response for the induction of NPY and SOM. It suggests that NT-3 in vivo presumably acts through the *trkB* receptor. Since expression of *trkB* and *trkC* is largely overlapping among cortical layers (Lamballe et al., 1994), it is difficult to assess contribution of individual neurotrophin receptors to our results. Answering this question will require careful quantitative and qualitative analyses for dose-responsiveness of neurotrophins in vivo and by combinatorial injections of the neurotrophins.

Immunohistochemistry for NPY, SOM, and SP revealed the same general theme, a comparable spatial pattern of peptide immunoreactivities among the various neurotrophins except NGF (Fig. 4 for NPY, Fig. 5 for SP, and Fig. 6 for SOM). The administration of BDNF, NT-3, and NT-5 resulted in the appearance of intense NPY-immunoreactive (NPY-IR) cells in the frontal and parietal neocortex in layers IV–VI as previously described (Nawa et al., 1994). SP-IR pyramidal neurons were abundant in layers II–III as well as in deeper layers of the BDNF and NT-3-injected brains (Fig. 5A), but rarely found in NGF or saline-treated animals. The effects of NT-5 could also be attributed to an increase in SP-IR in the upper layers (Fig. 5B), although it was sometimes difficult to distinguish real increases in SP immunoreactivity in soma and in apical dendrites. In contrast to our previous report (Nawa et al., 1994), we found more SOM-IR neurons induced by the neurotrophins in the present experiment, which extended from layer IV to layer VI in the frontal and parietal neocortex (Fig. 6B,C). This is the result of an optimized fixation that improved the sensitivity of our immunostaining with all antisera. In contrast, NGF (Fig. 6A) had no effect on SOM-IR cells, which were rare and found in layer VI in untreated or saline-injected animals. It remains to be elucidated how endogenous BDNF, NT-3, and NT-5 contribute to the peptidergic differentiation of each cortical layer in vivo. In addition, ligand-induced phosphorylation of *trks* in the cortex, striatum, and hippocampus suggests that the neurotrophins can activate in vivo their respective receptors as early as embryonic d 15 in the striatum and cortex (Knüsel et al., 1994). A clearer understanding of these interactions awaits the colocalization of neuropeptides and the receptors, including truncated forms of *trkB* and *trkC* as well as ligands during development.

In the last set of experiments, BDNF was administered directly into adult rat neocortex, hippocampus, and striatum using implanted osmotic pumps (Croll et al., 1994). Neuronal responses to BDNF in adult brain were significantly different from those seen in neonates. First, the magnitudes of SOM increases were small or negligible in most of the brain regions examined. Second, the neuropeptide induction was quite limited to nonpyramidal neurons. Third, the relative increase in NPY levels over control values was two- to threefold, which is much smaller than that observed in neonatal rats, although the absolute amounts of the NPY increases were almost comparable with the ones obtained in neonatal brain. The higher basal levels of NPY in adult rats made its relative increase look smaller. All those experiments indicate that neurotrophins regulate and/or maintain the phenotypic differentiation of neurotransmitter/peptide in the developing brain as well as in the mature brain.

BDNF Expression Is Regulated by Excitatory Neurotransmission

Our observations have suggested that neurotrophins, particularly BDNF, markedly

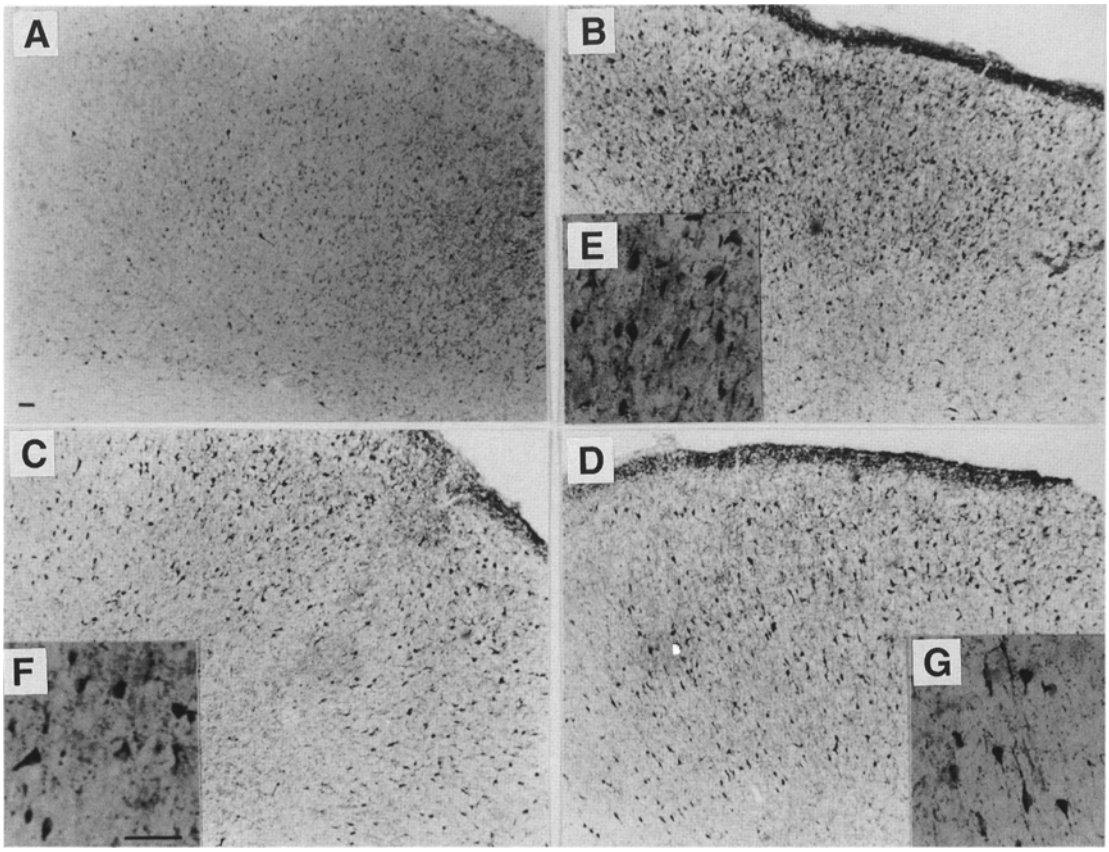


Fig. 4. NPY immunoreactivity in neocortex following neurotrophin injections. Forty-eight hours after intraventricular injections at postnatal d 3 of **(A)** NGF, **(B)** BDNF, **(C)** NT-3, and **(D)** NT-5, the rats were perfused with 4% paraformaldehyde and processed for cryotomy and immunostaining with antiserum against NPY at a dilution of 1:500. After incubating the sections with biotinylated antirabbit IgG antibodies followed by avidin–biotin complex, the immunoreactivity was visualized with peroxidase/VIP substrate reaction (Vector). (A–D) show sections of the right anterior neocortex taken around the site of insertion of cannula (visible in C). Note that layer I in BDNF (B), NT-3 (C), and NT-5 (D) is invaded by radially oriented fibers immunopositive for NPY. **(E–G)** Higher magnification of layer V where the density of NPY-IR neurons is dramatically enhanced by the injection of neurotrophins. All bars in (A–G) represent 50 μm.

change neurotransmitter/peptide expression both in vivo and in vitro. What is the upstream factor that controls BDNF expression itself? Previous experiments have shown that the excitatory neurotransmitter, glutamate, increases expression of BDNF mRNA as well as NGF mRNA in cultured hippocampal, neocortical, and cerebellar neurons through different types of glutamate receptors (Zafra et al., 1990, 1991; Bessho et al., 1993). Similarly, brain seizures induced by kainate injection or electrical stimulation elevate the expression of these mRNAs and their proteins (Gall and Isackson, 1989; Isackson et al., 1991; Dugich-Djordjevic et al., 1992; Nawa et al., 1995). The increase of BDNF mRNA is most remarkable in the neocortex and hippocampus, but disappears a few days later (Isackson et al., 1991; Dugich-Djordjevic et al., 1992). Not only such abnormal neuronal excitation but also physiological levels of neural impulses influence the basal level of BDNF mRNA. Light stimuli upregulate BDNF mRNA levels but not NGF mRNA levels in the rat visual cortex (Castren

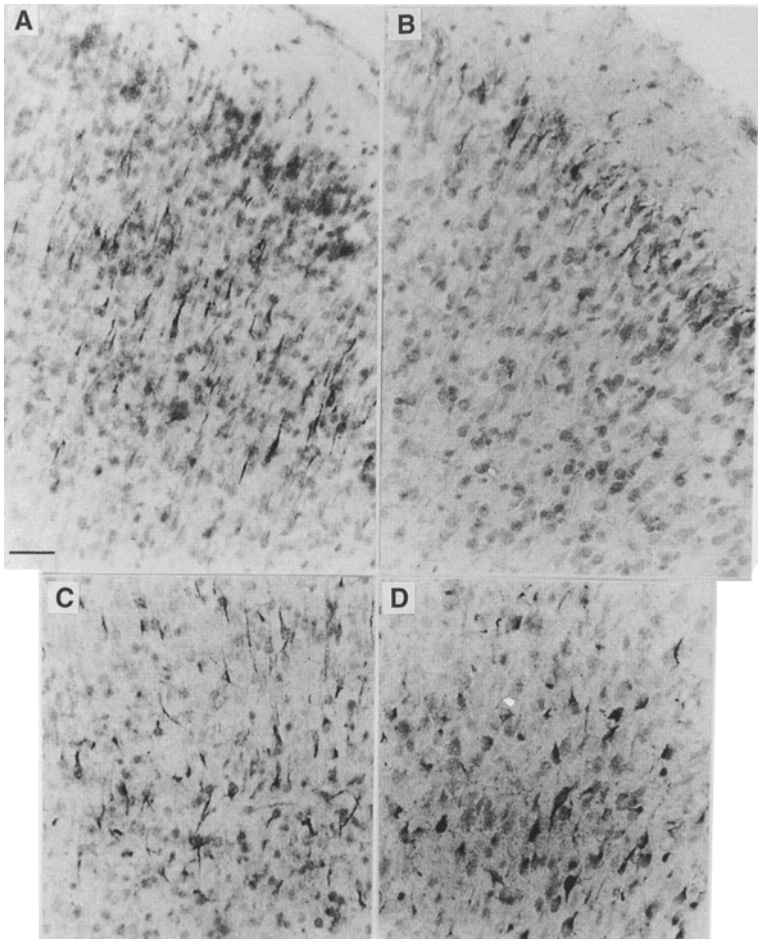


Fig. 5. SP-IR neurons induced by BDNF, NT-3, or NT-5 are found through layers of the cortex. Coronal sections through the parietal neocortex of rat receiving BDNF **(A,C)**, NT-5 **(B)**, or NT-3 **(D)** were immunostained with anti-SP antiserum. The treatments of BDNF and NT-3 increased the frequency of SP-IR neurons in layer II-III, whereas NT-5 effects seems restricted to layer II (A,B). The injection of the neurotrophins, except NGF, also resulted in SP-IR neurons with a large nucleus in layer V and VI (C for BDNF; D for NT-3). All bars represent 50 μm.

et al., 1992). Enhanced neurotransmission known as LTP can also elevate levels of BDNF mRNA in the hippocampus (Patterson et al., 1992; Castren et al., 1993). These observations strongly suggest that neuronal activity regulates BDNF expression in a plastic manner in the CNS (Lu et al., 1991). The dynamic and reversible responses of the mRNA expression indicate that BDNF as well as some other neurotrophins might be involved in important physiological reactions of the nervous system to many external stimuli.

Potential Physiological Significance of Neuropeptide Regulation by Neurotrophins

Does the production of BDNF that accompanies convulsive neuronal activities lead to neuropeptide induction? Several neurochemical studies on seizures have given hints on this question. It was reported previously that convulsive shock and brain seizures elevate expression of NPY, SOM, SP, and GABA (GAD)

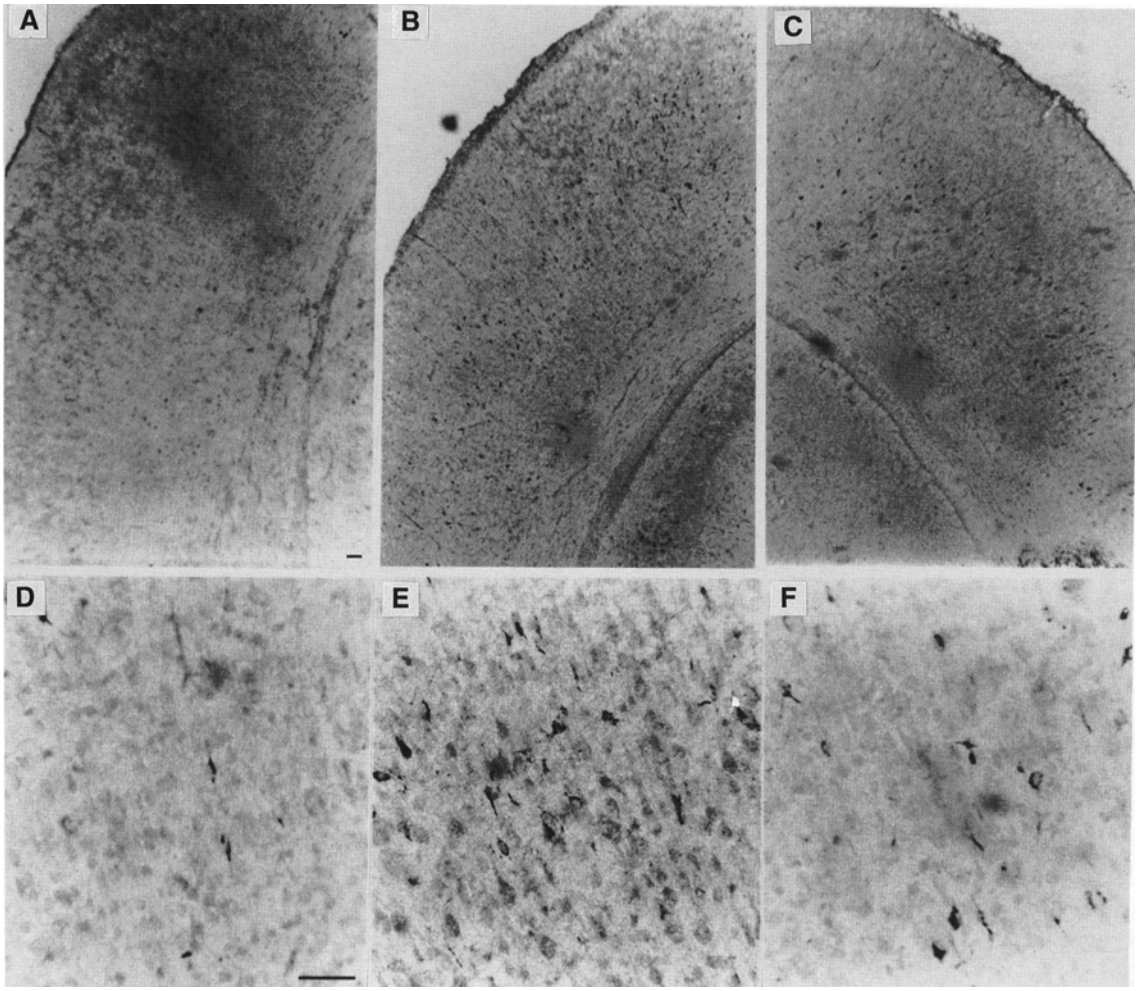


Fig. 6. Distribution of SOM-IR neurons after neurotrophin administration. Sections from parietal neocortex were immunostained with an antiserum against SOM. **(A)** Section from an animal treated with NGF. We did not detect any different distribution or frequency of SOM-IR cells when compared to saline-injected or noninjected rat at the same stage of development. **(B)** Equivalent section from an animal treated with BDNF and **(C)** NT-5. One can observe many ectopic SOM-immunoreactive neurons in layer IV–VI. **(D)** Typical SOM-IR neurons found in layer VI in NGF-treated rat, compared to the increased frequency of these SOM-IR cells in **(E)** NT-3 and **(F)** NT-5 in the same layer. All bars represent 50 μm.

in various brain regions (Sperk et al., 1986; Marksteiner and Sperk, 1988; Olenik et al., 1989; Bellmann et al., 1991; Lindefors et al., 1991). The neuropeptides induced by these neuronal activities are identical to those affected by neurotrophins as described here. Since BDNF mRNA is increased following kindling and brain seizures, but not NT-3 nor NT-5 (Isackson et al., 1991; Dugich-Djordjevic et al., 1992; Smith et al., 1993a), it is likely that BDNF, at least in part, mediates the peptidergic induction after seizures. In fact, the onset of BDNF mRNA increase after seizures precedes that of NPY mRNA increase by a few hours (Gall et al., 1991). These observations imply that excitatory neurotransmission mediated by glutamic acid stimulates the production of BDNF, which may subsequently increase the expression of

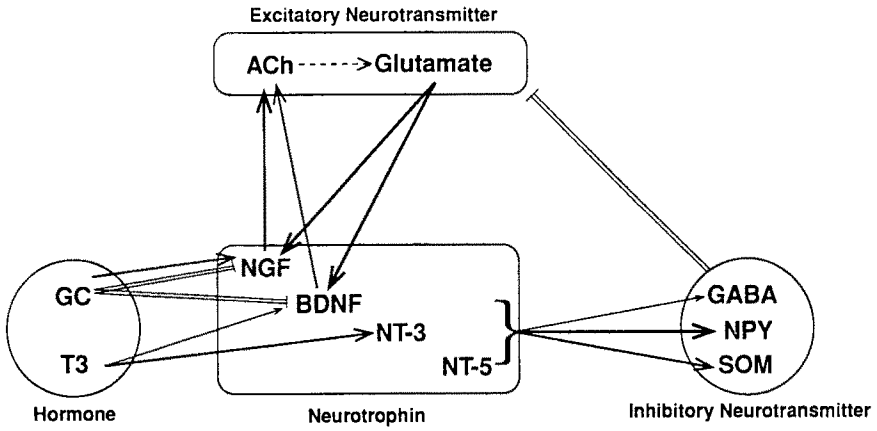


Fig. 7. Provisional interaction among neurotrophins, inhibitory, and excitatory neurotransmitters and hormones. Arrows represent enhancing synthesis and/or release, with increasing potencies symbolized by arrow thickness. The parallel lines terminated by line represent an inhibiting activity on synthesis and/or release. T3, Tri-iodothyronine; ACh, acetylcholine; GC, glucocorticoids.

NPY, SOM, SP, and GABA. Further studies will verify if this is indeed the case. A reversed relationship among neuronal activity, BDNF, and neuropeptides can be seen in patients with Alzheimer disease: Alzheimer disease is often associated with significant reduction in BDNF mRNA levels and various neurotransmitter/peptide contents, including acetylcholine, SOM, and NPY (Morrison et al., 1985; Kowall and Beal, 1988; Phillips et al., 1991). However, it cannot rule out that other factors, such as basic fibroblast growth factor (bFGF) and CDF/LIF, which can be induced or released by neuronal activity, may also contribute to the peptidergic changes (Burga et al., 1994; Gall et al., 1994; Habecker and Landis, 1994).

What are the physiological functions of the neurotransmitter and neuropeptides affected? One shared property by GABA, SOM, and NPY is to suppress neuronal excitability as inhibitory neurotransmitters or neuromodulators (Jacquin et al., 1985; Moore et al., 1994; Illes and Regenold, 1990; McQuiston and Colmers, 1993). GABA is a major inhibitory neurotransmitter that produces fast and slow inhibitory postsynaptic potential (IPSP) on postsynaptic neurons. NPY is thought to presynaptically inhibit excitatory neurotransmission in the hippocampus (McQuiston and Colmers, 1993). SOM elicits hyperpolarization of hippocampal pyramidal neurons by increasing potassium M-current (Moore et al., 1988). Therefore, these neurotransmitter and neuromodulators could serve as negative feedback regulators against the initial excitatory inputs.

The expression of NT-3 is enhanced by thyroid hormone T3 (Giordano et al., 1992; Lindholm et al., 1993). The synthesis of NGF in neurons and glial cells is regulated positively and negatively by glucocorticoids (Barbany and Persson, 1992; Lindholm et al., 1992). The production of acetylcholine is regulated by NGF and BDNF (Barde et al., 1982; Hatanaka and Tsukui, 1986; Murphy et al., 1991; Knüsel et al., 1992; Martinou et al., 1992). In turn, cholinergic activity upregulates the expression of NGF and BDNF in the hippocampus by influencing glutamatergic neurotransmission (Berzaghi et al., 1993). Glucocorticoids can downregulate BDNF expression in normal hippocampus (Smith et al., 1993b) as well as after kainic acid administration (Barbany and Persson, 1993). If we integrate all these relationships among excitatory neurotransmitters, neurotrophins, inhibitory neurotransmitters, and hormones, it emerges in Fig. 7 that, in the nervous system, NGF could positively control neuronal excitability via acetylcholine synthesis since acetylcholine enhances neuronal excitability (Krnjevic, 1975). Conversely, a major

excitatory neurotransmitter, glutamate, increases the production of NGF and BDNF. In contrast, BDNF as well as NT-3 and NT-5 would enhance inhibitory neurotransmission by increasing GABA, NPY, and SOM synthesis. This pathway could provide a negative feedback signal to the excitatory loop. Another level of regulation results from interactions with the endocrine system that also change the expression of all neurotrophins. All of these components could influence one another to regulate synthesis and release of neurotransmitter/neuropeptide and equilibrate the balance between excitatory and inhibitory neurotransmission in the CNS.

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