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Neuropeptide Messenger Plasticity in the CNS Neurons Following Axotomy

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Abstract

Neuronal peptides exert neurohormonal and neurotransmitter (neuromodulator) functions in the central nervous system (CNS). Besides these functions, a group of neuropeptides may have a capacity to create cell proliferation, growth, and survival. Axotomy induces transient (1–21 d) upregulation of synthesis and gene expression of neuropeptides, such as galanin, corticotropin releasing factor, dynorphin, calcitonin gene-related peptide, vasoactive intestinal polypeptide, cholecystokinin, angiotensin II, and neuropeptide Y. These neuropeptides are colocalized with "classic" neurotransmitters (acetylcholine, aspartate, glutamate) or neurohormones (vasopressin, oxytocin) that are downregulated by axotomy in the same neuronal cells. It is more likely that neuronal cells, in response to axotomy, increase expression of neuropeptides that promote their survival and regeneration, and may downregulate substances related to their transmitter or secretory activities.

Index Entries: Neuropeptides; neuropeptide mRNA; axotomy; central nervous system; neuronal plasticity.

Introduction

During the past two decades, increasing numbers of neuropeptides have been identified in the central nervous system (CNS). More than 40 neuropeptides, ranging in size from the dipeptide *N*-acetyl-aspartylglutamate to molecules over 30 amino acids in length, are expressed in the brain. Immunohistochemical and neurochemical studies have demonstrated that each of these neuropeptides has its own individual distribution pattern throughout the CNS (*see* Palkovits, 1988). Neurons once thought to use amino acids, biogenic amines, or acetylcholine as neurotransmitters have also been shown to synthesize at least one neuropeptide, and a great deal of evidence shows that neuronal cells can produce even more neuropeptides. Colocalization of these substances in the same neurons seems to be a widely accepted principle (*see* Hökfelt et al., 1987b, 1994; Hökfelt, 1991). The colocalized neuropeptides and neurotransmitters are packaged in large secretory granules, stored in

nerve terminals and varicosities, and secreted on appropriate stimuli.

Neuronal peptides are expressed in the brain during the early ontogenesis. Some of them remain permanently expressed in specific brain regions, whereas others are only transiently expressed, i.e., their synthesis declines dramatically or seemingly disappears. In extreme conditions (injury, axotomy, extreme changes in water and electrolyte milieu, hypophysectomy, adrenalectomy, or serious stressful situations, and so on), however, these neuronal cells are able to synthesize neuropeptides.

It has been proposed that, in general terms, neuropeptides have two major physiological roles, as neurohormones and as neurotransmitters.

1. Neurohormonal role: After their release from nerve terminals or varicosities into the circulation, neuropeptides reach their target organs (endocrine glands) and exert their effects extraneuronally. During the past decades, an involvement in neuroendocrine events for several neuropeptides has been demonstrated (*see* McCann, 1982, Hökfelt et al., 1987a).
2. Neurotransmitter role: Neuropeptides are stored in synaptic vesicles, and then released from synaptic boutons and exert their effects on the postsynaptic site, influencing the activity of another neuron. In many cases, neuropeptides in certain neurons (mainly in the hypothalamus) may exert their neurohormonal and neurotransmitter roles simultaneously: Some of the axon collaterals may synapse on other neurons, whereas other collaterals of the same axon terminate on the pericapillary space in specific brain regions (median eminence, circumventricular organs) or in the posterior pituitary (Csiffáry et al., 1992).

Several neuropeptides have been shown to exert functions other than neurohormonal or neurotransmitter to regulate cell proliferation, growth, and survival (*see* Hökfelt, 1991; Schwartz, 1992). During the past several years, there has been a great deal of interest in the effect of axotomy on neuropeptide synthesis and gene expression in lesioned neuronal cells. It is of particular interest that axotomy induces the expression and synthesis of certain neuropeptides and, at the same time, the expression and synthesis of neurotransmitters that are colocalized in the same neuronal cell are depressed. Information obtained using the axotomy paradigm in several laboratories, including our own, are summarized herein.

Axotomy-Induced Changes in CNS Neurons

Upregulated Neuropeptide Synthesis and Gene Expression

Corticotropin Releasing Factor (CRF)

Corticotropin releasing factor (CRF) is highly concentrated in the hypothalamo–hypophyseal system. Neurons in the parvicellular paraventricular nucleus are the major sources of CRF, which is stored in the median eminence, and after a release into the portal circulation, it acts as a neurohormone by releasing ACTH from the anterior pituitary. CRF is widely distributed in the CNS, stored in synaptic vesicles, and exerts its action as a neurotransmitter or neuromodulator on other neuronal cells. In certain brain regions, CRF is colocalized with "classic" neurotransmitters. In the inferior olive, CRF is cosynthesized with aspartate and is present in the climbing fibers in the olivocerebellar system (Palkovits et al., 1987). After unilateral transection of the olivocerebellar tract, which resulted in a depletion of the aspartate level in the contralateral inferior olive cells (fibers in the olivocerebellar tract crossover at the level of the inferior olive, completely), CRF was dramatically upregulated contralateral to the knife cut (Fig. 1). The marked increase in levels of CRF peptide and CRF mRNA content in the inferior olive was observed a few hours after axotomy (Mezey and Palkovits, 1991).

CRF mRNA accumulated in the prepositus hypoglossal nucleus after a unilateral transection of the inferior cerebellar peduncle (Fig. 2).

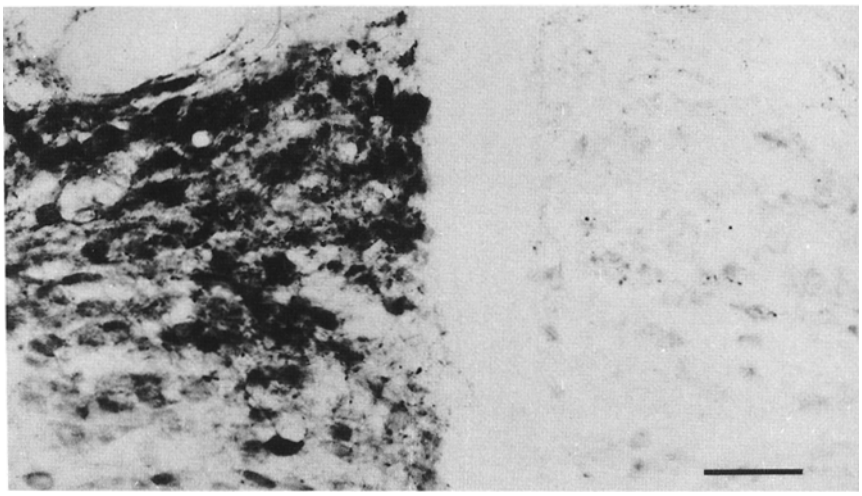

Fig. 1. Principal nucleus of the inferior olive in the rat, coronal section. Corticotropin-releasing factor (CRF)-like immunoreactivity in the nucleus 3 d after unilateral transection of the olivocerebellar tract, contralateral to the knife cut. (The side of the transection is labeled with a star.) Abbreviations: R, medullary raphe. Bar scale: 50 μm.

In intact rats (or contralateral to the knife cut), CRF expression is almost undetectable there (Fig. 2A). The "classic" neurotransmitter of the prepositus neurons is not yet known.

Under "normal" conditions, CRF is present in the dorsal vagal complex, but only in neurons of the nucleus of the solitary tract. After unilateral vagotomy (both in intracranial or cervical transections), there were no visible changes either in CRF synthesis or CRF gene expression in the nucleus of the solitary tract, but a marked upregulation was found in CRF mRNA in the dorsal motor vagal nucleus ipsilateral to the knife cut (not shown).

It is worth mentioning that brain regions where CRF is the apparent "dominant" neuropeptide, such as in the hypothalamic paraventricular nucleus, axotomy resulted in a downregulation of this neuropeptide (Palkovits et al., unpublished observation).

Galanin

This neuropeptide is widely distributed throughout the CNS. It is upregulated dramatically in various neuronal cell types a few hours after axotomy. One to seven days after a unilateral knife cut of the olivocerebellar tract, accumulation of galanin peptide (Fig. 3A) and preprogalanin mRNA (Fig. 3B) were observed in neuronal perikarya of the principal nucleus of the inferior olive contralateral to the transection (fibers completely crossover at the level of the inferior olive). After 7 d of axotomy there was a marked reduction in the number of galanin immunoreactive or galanin mRNA-labeled neurons in the inferior olive, and they disappeared by the 21st postoperative day. Neurons on the ipsilateral side remained unlabeled during the whole period investigated (Fig. 3C). Unilateral intracranial transection of the hypoglossal nerve induced increased galanin immunoreactivity (Palkovits and Horváth, 1994) and levels of preprogalanin mRNA in the motor hypoglossal nucleus ipsilateral to the knife cuts (Fig. 4). Neurons in the contralateral nucleus remained completely unlabeled. Axotomy-induced increases in galanin have also been reported in neurons of the motor facial nucleus (Moore, 1989; Saika et al., 1991). Transient (2–3 wk after axotomy) upregulation of galanin gene expression was observed within subpopulations of spinal cord

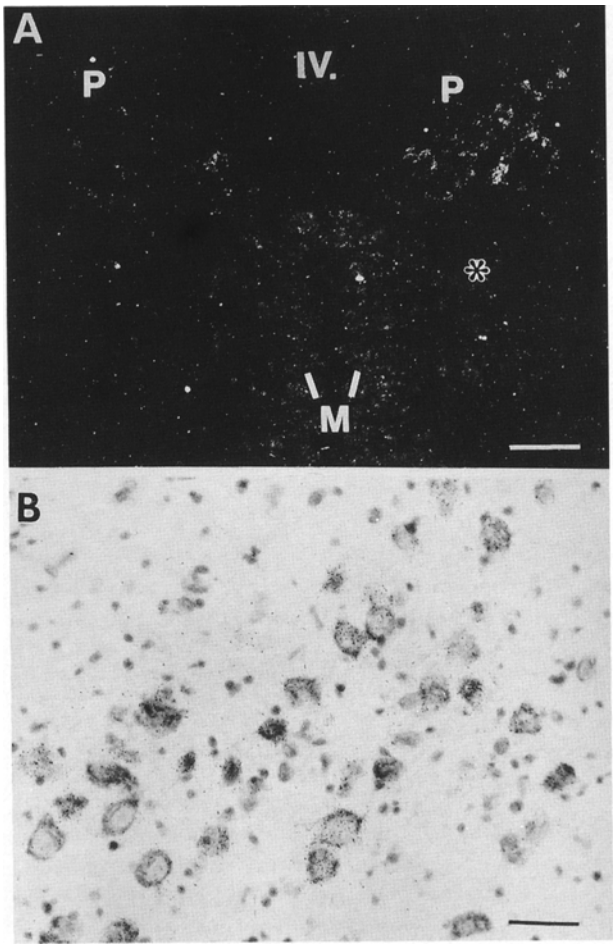

Fig. 2. Prepositus hypoglossal nucleus (P) in the rat, coronal sections. **(A)** Corticotropin-releasing factor (CRF) mRNAs in the nucleus. One day after a unilateral transection of the inferior cerebellar peduncle. (The side of the transection is labeled with a star.) Labeled cells are almost exclusively ipsilateral to the knife cut. **(B)** High-power magnification of CRF-labeled cells in the ipsilateral prepositus hypoglossal nucleus. Abbreviations: M, medial longitudinal fascicle; IV, fourth ventricle. Bar scales: (A) 200 μm; (B) 50 μm.

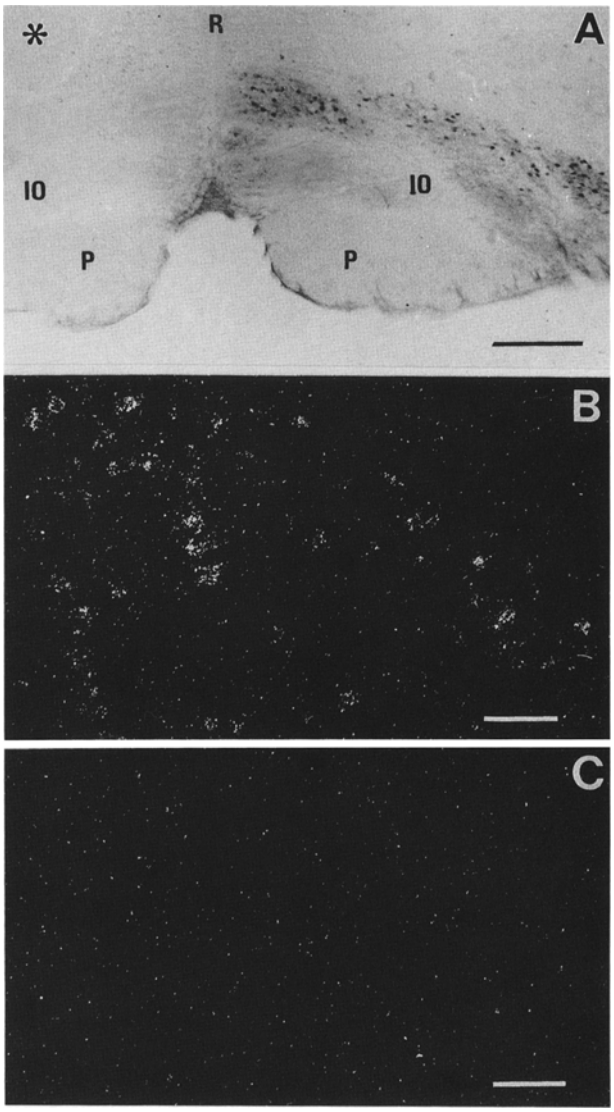

Fig. 3. Principal nucleus of the inferior olive in the rat, 3 d after unilateral transection of the olivocerebellar tract (coronal sections). **(A)** Galanin-like immunoreactivity in the nucleus contralateral to the knife cut, whereas the ipsilateral side is immunonegative. (The side of the transection is labeled with a star.) **(B)** Preprogalanin (ppGAL) mRNA in inferior olivary neurons, contralateral to the knife cut. **(C)** No ppGAL labels are seen in the ipsilateral inferior olive. Abbreviations: IO, inferior olive; P, pyramidal tract; R, medullary raphe. Bar scales: (A) 500 μm; (B and C) 100 μm.

motoneurons ipsilateral to the axotomy (Zhang et al., 1993).

Four days after unilateral axotomy (intracranial or cervical vagotomy), galanin immunoreactivity (Fig. 5) and preprogalanin mRNA (Fig. 6) appeared in cells of the dorsal motor vagal and ambiguus nuclei ipsilateral to the knife cut. By the 7th postoperative day, six- to tenfold increases in the level of preprogalanin mRNA were measured in these nuclei by Rutherfurd et al. (1992).

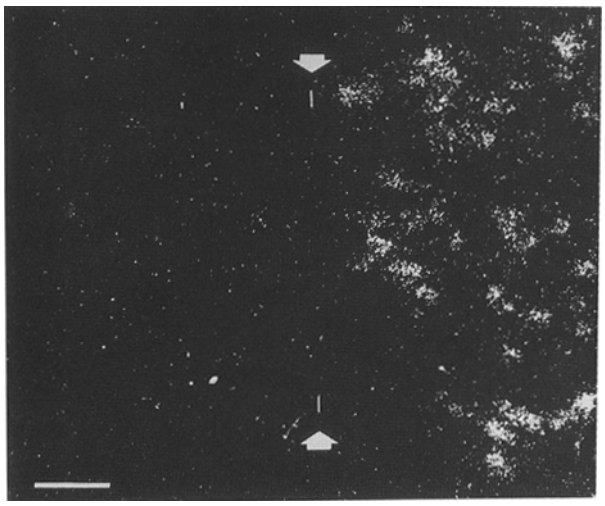

Fig. 4. Preprogalanin mRNA-labeled cells in the motor hypoglossal nucleus, ipsilateral to the transection of the roots of the hypoglossal nerve. The contralateral side is completely unlabeled (the midline is indicated by two arrows). Bar scale: 100 μm.

In the nucleus of the solitary tract, which is the sensory component of the dorsal vagal complex, vagotomy did not affect galanin gene expression (Fig. 6), indicating that transection of vagal efferents (from the dorsal vagal and ambiguus nuclei) but not vagal afferents (fibers to the nucleus of the solitary tract) induces upregulation of galanin mRNA in neurons of the dorsal–vagal complex.

The expression of galanin is enhanced after cerebral decortication (frontoparietal cortex) in the dorsal raphe nucleus, the dorsal and ventral thalamic nuclear groups, and in the cingulate and piriform cortex (Cortés et al., 1990b). Ventral hippocampal lesions (Cortés et al., 1990b), a surgical transection or chemical lesioning (tetradotoxin) of the septohippocampal pathway (Ágoston et al., 1994), markedly increase galanin mRNA in the medial septal nucleus–nucleus of the diagonal band complex.

Hypophysectomy (by lesioning the fibers of the hypothalamo–hypophyseal tract) enhanced galanin synthesis (Selvais et al., 1993) and galanin expression (Arvidsson et al., 1989; Villar et al., 1990; Young et al., 1990) in the hypothalamic magnocellular neurons. Axotomy in the hypothalamo–hypophyseal tract at the retrochiasmatic area resulted in a similarly increased galanin synthesis and gene expression in the supraoptic (Young et al., 1990) and paraventricular nucleus (Fig. 7).

Dynorphin

A significant increase both in the number and intensity of dynorphin-immunoreactive cells in the spinal cord laminae I, II, and V occurs after rhizotomy (Cho and Basbaum, 1988). Upregulation in dynorphin synthesis and gene expression have also been reported in the lumbar spinal cord caudal to a lesion in the thoracic cord (Faden et al., 1985).

Four days after axotomy (unilateral transection of the hypothalamo–hypophyseal tract), dynorphin-immunoreactive material (Fig. 8A) and preprodynorphin mRNA (Fig. 8B) appeared in the cells of the supraoptic nucleus ipsilateral to the knife cut. These increases peaked at 7 d, and lasted up to 3 wk. Hypophysectomy resulted in dynorphin-immunopositivity in magnocellular neurons of the supraoptic and paraventricular nuclei at 1 d after removal of the gland (Villar et al., 1990).

Calcitonin Gene-Related Peptide (CGRP)

A rapid elevation in the expression of CGRP mRNA was found in axotomized motoneurons (peripheral nerve section or ventral root transection) in the spinal cord (Arvidsson et al., 1990; Piehl et al., 1991). Motoneurons in the cranial nerve nuclei also express increased levels of CGRP mRNA in response to axotomy (Moore, 1989; Streit et al., 1989; Haas et al., 1990, 1993; Marlier et al., 1990; Dumoulin et al., 1991; Saika et al., 1991). Worthy of note is that axotomy increased only the transcription of α-CGRP whereas the β-CGRP mRNA level was decreased after axotomy (Noguchi et al., 1990; Saika et al., 1991).

Vasoactive Intestinal Polypeptide (VIP)

Following axotomy, a small number of VIP mRNA-positive motoneurons were observed

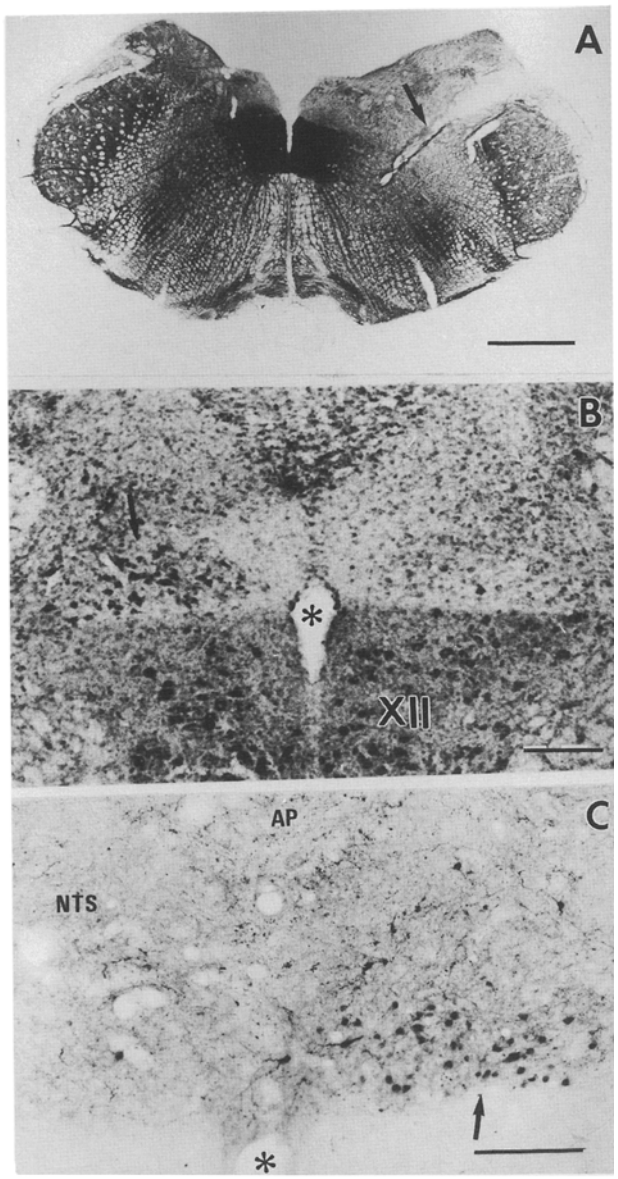

Fig. 5. Unilateral intracranial vagotomy in rats. Coronal sections. **(A)** By the 14th postoperative day, the strong acetylcholinesterase staining disappeared from the dorsal motor vagal nucleus ipsilateral to knife cut (arrows), whereas it remained unchanged on the contralateral side or on both sides of the motor hypoglossal nucleus. **(B)** Choline acetyltransferase immunostaining. Disappearance of staining from the ipsilateral dorsal motor vagal nucleus, 4 d after vagotomy. **(C)** Galanin-like immunostaining in dorsal motor vagal neurons ipsilateral to the knife cut, 4 d postoperatively. Abbreviations: AP, area postrema; D, dorsal motor vagal nucleus; H, motor hypoglossal nucleus; N, commissural part of the nucleus of the solitary tract; S, spinal trigeminal nucleus; *, central canal. Bar scales: (A) 1 mm; (B) 100 μm; and (C) 200 μm.

in the ventral horn of the spinal cord (Zhang et al., 1993). Reportedly, the highest percentage of labeled neurons was found at 7 d, after which they decreased in number or disappeared by the third postoperative week. Brainstem hemisection also resulted in upregulation of VIP mRNA in some midbrain (nucleus interstitialis Cajal) and medullary (peritrigeminal) nuclei (Palkovits et al., in preparation).

Angiotensin II

Three days after unilateral cervical or intracranial vagotomy, angiotensin II-immunoreactive neurons appeared in the dorsal motor vagal nucleus and in the rostral part of the ambiguus nucleus ipsilateral to the transaction. No immunoreactivity was seen in the nucleus of the solitary tract. The intracranial portion of the vagus nerve showed strong angiotensin II -immunoreactivity proximal to the knife cut (Palkovits and Fodor, unpublished observation).

Cholecystokinin (CCK)

Enhanced CCK mRNA was observed in facial motoneurons 1 d after axotomy. From the third postoperative day, the level of CCK mRNA gradually increased and remained elevated for 2 wk (Saika et al., 1991). In hypophysectomized rats, magnocellular supraoptic and paraventricular neurons showed CCK upregulation that reached the highest level 5–7 d after operation. Since CCK is principally colocalized with oxytocin, the changes in CCK mRNA levels are likely to reflect an activation in oxytocin-containing magnocellular neurons (Villar et al., 1990).

Neuropeptide Y (NPY)

Upregulation of NPY mRNA was observed within subpopulations of spinal cord motoneurons ipsilateral to axotomy up to 4 wk after surgery (Zhang et al., 1993). Transections of trigeminal axons in the brainstem resulted in

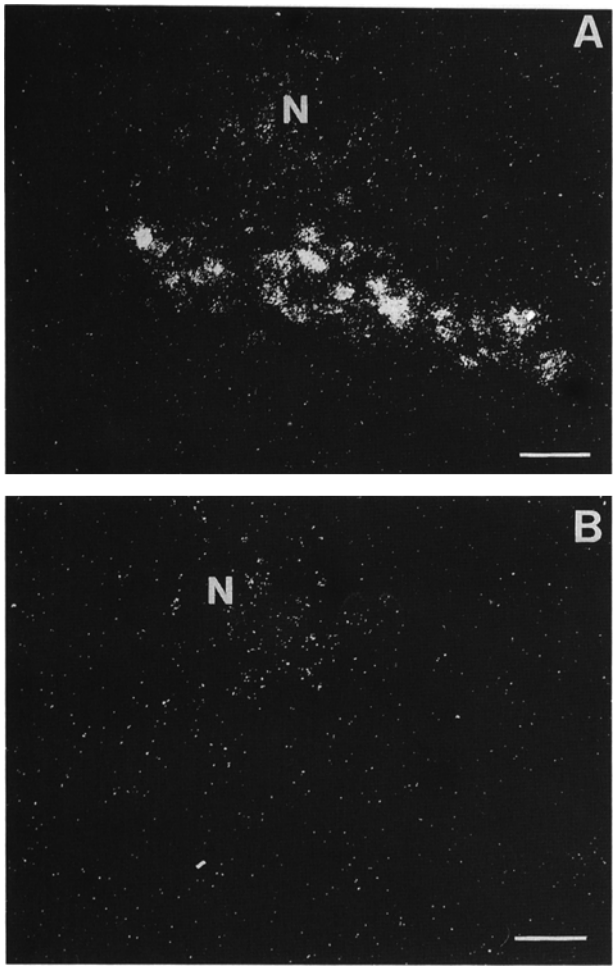

Fig. 6. Preprogalanin mRNA in the dorsal motor vagal nucleus ipsilateral to the unilateral intracranial vagotomy (*see* Fig. 5A), 4 d after surgery. **(A)** No labels are seen in the contralateral dorsal motor vagal nucleus. **(B)** The light labeling in the nucleus of the solitary tract (N) did not change after vagotomy. Bar scales: 100 μm.

an accumulation of NPY in neuronal perikarya of the paratrigeminal nucleus (Fodor and Palkovits, 1991). In contrast to these, brainstem hemisection did not alter NPY synthesis or gene expression in brainstem catecholaminergic neurons which cosynthesize NPY.

Substance P

Axotomy induces transient (2–7 d) expression of substance P mRNA in spinal cord motoneurons (Zhang et al., 1993).

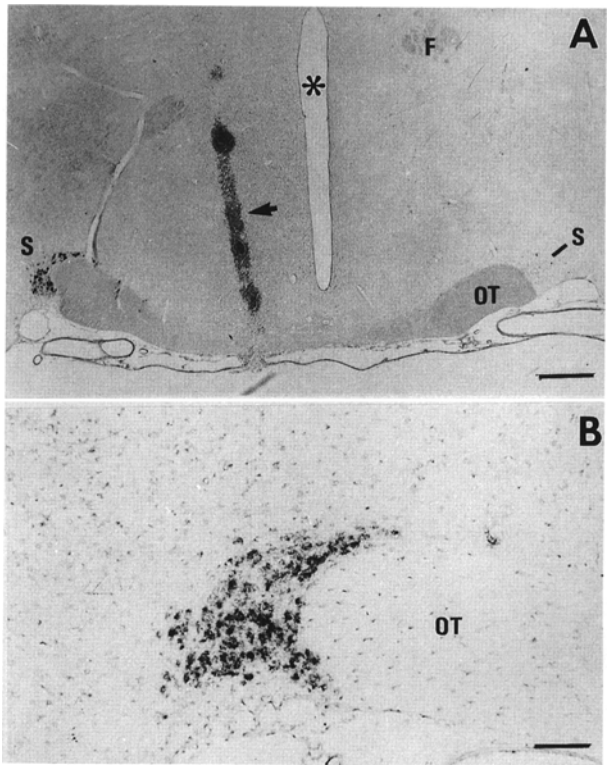

Fig. 7. Unilateral transection of the hypothalamo–hypophyseal tract (*see* Fig. 8A). Coronal sections, 4 d after surgery. **(A)** Strong galanin immunostaining in the magnocellular portion of the paraventricular nucleus (and in few parvicellular neurons) ipsilateral to the knife cut, whereas the contralateral nucleus remained unstained. **(B)** Preprogalanin mRNA labeling in the ipsilateral paraventricular nucleus. Abbreviation: *, third ventricle. Bar scales: 200 μm.

Somatostatin

Upregulation of somatostatin gene expression in spinal cord motoneurons has been reported following axotomy (Zhang et al., 1993). In contrast to this finding, Rutherfurd et al. (1992) did not find any evidence for upregulation of somatostatin mRNA levels in the dorsal motor vagal nucleus after cervical vagotomy. In the hypothalamic periventricular nucleus, where somatostatin is present in neurons projecting to the median eminence, axotomy downregulates somatostatin synthesis and gene expression (Palkovits et al., unpublished observation).

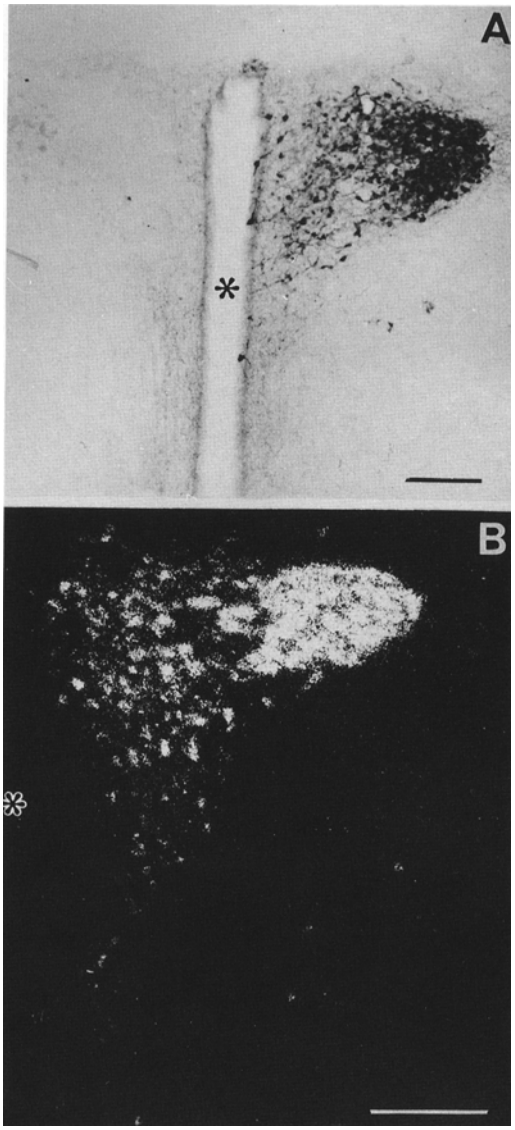

Fig. 8. Unilateral transection of the hypothalamo–hypophyseal tract (arrow) at the retrochiasmatic level in the rat. Coronal sections 4 d after surgery. **(A)** Dynorphin B immunoreactive cells in the supraoptic nucleus ipsilateral to the knife cut, whereas no staining is visible on the contralateral nucleus. **(B)** Preprodynorphin mRNA in supraoptic neurons ipsilateral to the transections. Abbreviations: F, fornix; OT, optic tract; S, supraoptic nucleus; *, third ventricle. Bar scales: (A) 500 μm; (B) 100 μm.

Enkephalin

Data available in the literature are somewhat contradictory regarding enkephalin synthesis and gene expression in response to axotomy. In our experiments, upregulation in enkephalin synthesis or gene expression did not occur after any type of axotomy. Similarly, Rutherfurd et al. (1992) did not find any evidence for upregulation of enkephalin mRNA levels in the dorsal motor vagal nucleus after cervical vagotomy, nor did Cho and Basbaum (1988) in the spinal cord after rhizotomy. In contrast to these negative observations, increased enkephalin-immunoreactivity in spinal motoneurons was reported by Linda et al. (1990) after axotomy, whereas a transient downregulation of enkephalin gene expression was found in such motoneurons by Zhang et al. (1993).

Time-Course of Axotomy-Induced Changes in Neuropeptide Synthesis and Gene Expression

Axotomy induces transient changes in neuropeptide synthesis and gene expression in CNS neurons. The first noticeable signs, as well as the duration of the changes, show marked variations depending on the phenotypes, the functional role, and the location of affected neurons in different brain areas or systems. In general, axotomy-induced alterations in peptide synthesis (immunoreactivity) are first noticed 1–2 d after lesion and they persist until the third or fourth postoperative week. This time period corresponds with the duration of the increased level of protein synthesis and energy metabolism in axotomized neurons. These later increases are first detected 24 h postaxotomy, they are maximal 1–3 d postoperatively, and persist 21–35 d (Smith et al., 1984).

Very early appearance was found for CRF (Mezey and Palkovits, 1991), CGRP (Streit et al., 1989), and dynorphin (Villar et al., 1990) immunoreactivity: 12–24 h after axotomy. They reached maximal intensity 3–6 d postoperatively and then they slowly declined.

The time course of changes in mRNA levels is different from the time course of changes in immunoreactivity. Changes in gene expression by axotomy may occur at an earlier time point

than previously thought. A slight increase in CRF mRNA levels was seen in inferior olive neurons 30 min after olivocerebellar transection, levels were then increased markedly 3 h postoperatively (Mezey and Palkovits, 1991). This observation is in good agreement with previous studies showing early changes in neuronal RNA content and nuclear proteins (Watson, 1968; Haddad et al., 1969; Buriani et al., 1990).

The levels of mRNAs of different neuropeptides return to the control values by different time periods following axotomy. These durations are strongly affected not only by the phenotypes, but by the type of the lesion (Saika et al., 1991). In general, the high level of CRF gene expression is maintained for 7 d only (Mezey and Palkovits, 1991), whereas CGRP (Piehl et al., 1991) and substance P (Zhang et al., 1993) mRNAs remained elevated 2 wk postoperatively. Galanin mRNA, which reached the maximum 3–6 d after axotomy, showed a marked reduction at 14 d of survival, and persisted no longer than 3–4 wk postoperatively (Villar et al., 1990; Young et al., 1990; Saika et al., 1991; Zhang et al., 1993). In contrast to listed neuropeptide mRNAs, the axotomy-induced high levels of CCK (Saika et al., 1991), NPY, and somatostatin (Zhang et al., 1993) mRNAs were maintained for a duration over 4 wk postoperatively.

Axotomy-Induced Simultaneous Changes in the Synthesis and Expression of Colocalized Neuropeptides and Neurotransmitters

Axotomy strongly influences the gene expression of active substances, which coexist in the same neuronal cell of the CNS. In general terms, changes following axotomy involve the downregulation of the apparent "dominant" neurotransmitter and upregulation of the "silent" colocalized neuropeptide(s). There may be a shift away from synthesis of materials required for neurotransmission or neurosecretion toward the production of structural proteins, such as tubulin, or of nerve growth factors, or of substances that may participate in the restitution or regeneration of lesioned neuronal cells. Some of the neuropeptides may belong to the class of these substances. Observations taken from various types of brain regions and systems after axotomy support this proposal (Table 1).

General Considerations: Possible Mechanism of Axotomy-Induced Upregulation in Neuropeptide Gene Expression

The mechanism of altered gene expression in axotomized neurons is not fully understood yet. It is still unclear what kind of signals induce certain neuropeptide gene expression of these neurons. Axon damage or blocking of axonal flow by colchicine (Cortés et al., 1990a) may prevent the transport of target-derived substances (i.e., growth factors) from the nerve terminals that may influence gene expression. This "lesion effect" may not be responsible exclusively for this phenomenon, since a blockade of neuronal activity by tetradotoxin alone can lead to temporary upregulation of neuropeptide gene expression (Ágoston et al., 1994).

The induction of cellular immediate early genes is among the earliest molecular events known so far that occur in CNS neurons in response to axotomy. These immediate early genes have been shown to encode transcription factors that have been proposed to activate target genes encoding neuropeptides (Sheng and Greenberg, 1990).

Axotomy is associated with the selective induction of immediate early genes *c-jun* and *jun D* but not *c-fos*. They trigger regeneration-associated alterations in neuropeptide gene expression in axotomized neurons. There is a clear temporal and spatial correlation between *c-jun* mRNA expression and the induction of CGRP gene in injured facial motoneurons (Haas et al., 1993), or dorsal motor vagal neurons (Herdegen et al., 1991). Recently, Brecht et

Table 1
Axotomy-Induced Simultaneous Changes in the Synthesis and Gene Expression of Colocalized Neurotransmitters and Neuropeptides of Various Functional Systems and Nuclei in the CNS

Functional systems	Brain nuclei investigated	Downregulated "dominating" phenotype	Upregulated "silent" phenotype	References
Septohippocampal	Medial septal and diagonal band nuclei	Acetylcholine	Galanin	Cortés et al. 1990b Ágonston et al.
Magnocellular hypothalamo–hypophyseal	Supraoptic and paraventricular	Vasopressin	Galanin	Villar et al., 1990 Young et al., 1990
		Vasopressin	Dynorphin	Villar et al., 1990[a]
		Oxytocin	CCK	Villar et al., 1990
Parvicellular hypothalamo–hypophyseal	Paraventricular	CRF	Galanin	[a]
Olivocerebellar	Inferior olive	Aspartate	CRF	Mezey and Palkovits, 1991[a]
		Aspartate	Galanin	[a]
Autonomic (parasympathetic)	Dorsal motor vagal and ambiguus	Acetylcholine	Galanin	[a]
		Catecholamine	Galanin	Rutherfurd et al.,
		Acetylcholine	CRF	[a]
		Acetylcholine	Angiotensin II	[a]
Serotoninergic	Dorsal raphe	Serotonin	Galanin	Cortés et al., 1990b
Cranial motor	Facial and hypoglossal	Acetylcholine	Galanin	Moore, 1989; Saika et al, 1991[a]
		Acetylcholine	CGRP	Moore, 1989; Streit et al., 1989; Haas et al., 1990, 1993; Marlier et al., 1990; Saika et al., 1991
Spinal motor	Ventral horn	Acetylcholine	Galanin	Zhang et al., 1993;
		Acetylcholine	Dynorphin	Cho and Basbaum, 1988
		Acetylcholine	VIP	Zhang et al., 1993
		Acetylcholine	NPY	Zhang et al., 1993

[a]Present study.

al. (1994) reported that *c-jun* is expressed in axotomized neurons in the absence of the calcium/cAMP response element-binding protein (CREB) transcription factor, which is downregulated following axotomy. The CREB protein is the nuclear target of CA^{2+} and cAMP-activated second messenger cascades, and acts on *c-jun* and genes encoding for effector proteins, such as certain neuropeptides or neurotransmitters synthesizing enzymes.

Although more than 40 neuropeptides are expressed in the CNS, only a limited number

of them exhibit "neurotrophic-like" action. Furthermore, these peptides are upregulated in response to axotomy only in certain brain regions, whereas in other regions or nuclei they remain silent. The axotomy-induced high level of peptide synthesis and gene expression is transient and their time courses show fairly large, phenotype-dependent variations. In most cases, they return to the "normal" level (in parallel with the return of downregulated coexpressed "dominant" neurotransmitters or neuropeptides) 2–3 wk after axotomy, although the neuronal cell is still axotomized and functionally paralyzed. This phenomenon and the functional significance of axotomy-induced upregulation in neuropeptide synthesis and gene expression remain to be elucidated.

Addendum: Materials and Methods

Axotomy

The following neuronal transections were performed in our laboratory:

1. Brainstem hemisection (Palkovits et al., 1992);
2. Pituitary-stalk transection (obtained from Civic Miller), lesions of the median eminence (Palkovits et al., 1989);
3. Fiber-transection in the lateral retrochiasmatic area (transection of the hypothalamo–hypophyseal tract) (Palkovits, 1982);
4. Cervical vagotomy;
5. Intracranial vagotomy (de Jong and Palkovits, 1975);
6. Intracranial transection of the hypoglossal nerve (Palkovits and Horváth, 1994);
7. Transection of the olivocerebellar tract (Mezey and Palkovits, 1991); and
8. Transection and chemical lesion of the septohippocampal pathway (Ágoston et al., 1994).

In addition to these, hypophysectomy (Arvidsson et al., 1989; Villar et al., 1990; Selvais et al., 1993), decortication (frontoparietal cortex ablation by aspiration) and ventral hippocampal lesions (Arvidsson et al., 1990; Cortés et al. 1990b), unilateral cervical vagotomy (Rutherfurd et al., 1992), spinal cord injury (Faden et al., 1985), or rhizotomy (Cho and Basbaum, 1988; Piehl et al., 1991); transection of the roots of the facial (Moore, 1989; Saika et al., 1991; Haas et al., 1993), oculomotor, and glossopharyngeal (Moore, 1989) nerves, septohippocampal lesion (Cortés et al., 1990b) were performed.

Immunohistochemistry

Adult male Wistar-Kyoto rats (weight 250–300 g) were used. Under ether anesthesia, animals were intracardially perfused with 50 mL saline followed by 500 mL fixative solution (4% paraformaldehyde, 0.19% picric acid in 0.1*M* phosphate buffer, pH 7.35). The brains were removed and postfixed. Forty-micrometer thick coronal sections were cut on a freezing microtome and processed for immunohistochemistry. The primary antibodies were visualized using the avidin-biotin-peroxidase technique on free-floating sections. The primary antibodies were used overnight at 4°C in various dilutions (CRF: 1:10,000, galanin: 1:10,000, dynorphin A: 1:2000, VIP: 1:20,000, NPY: 1:32,000, angiotensin II: 1:4000, vasopressin: 1:4000, somatostatin: 1:20,000) in PBS with 0.6% Triton and 5% normal goat serum. For the specificity of the antibodies, *see* Lantos et al. (1995).

In Situ *Hybridization Histochemistry*

Frozen, 12-μm thick sections were cut in a cryostat and mounted on gelatin-coated slides and kept at –80°C until used. Prior to hybridization, the sections were fixed in 4% formalin, rinsed, incubated with triethanolamine hydrochloride-containing acetic acid, dehydrated, and delipidated through a series of ethanols and chloroform. Then, the sections were air dried and covered with a hybridization buffer containing 10^6 cpm of [^{35}S]-labeled oligonucleotide probes. After an overnight incubation at 37°C, the sections were washed in 50% forma-

lin/2X SSC for 60 min at 40°C, then in 1X SSC for 2 h at room temperature and air-dried. The sections were coated with emulsion (Kodak NTB3) and developed for 1–3 wk. The procedure has been described in detail previously (Young et al., 1986).

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