Molecular Neurobiology

ISSN0893-7648/93/7(2): 87–110/$4.60

Gene Expression and Chemical Diversity in Hypothalamic Neurosecretory Neurons

Björn Meister

Department of Histology and Neurobiology, Karolinska Institute, PO Box 60400, 104 01 Stockholm, Sweden

Contents

Abstract

Hypothalamic neurosecretory neurons transcribe, translate, store, and secrete a large number of chemical messengers. The neurons contain hypothalamic signal substances that regulate the secretion of anterior pituitary hormones as well as the neurohypophysial peptides vasopressin and oxytocin. In addition to the classical hypophysiotropic hormones, a large number of neuropeptides and classical transmitters of amine and amino acid nature are present in the same cells. This is particularly evident in the magnocellular neurons of the supraoptic and paraventricular nuclei, and in parvocellular neurons of the arcuate and paraventricular nuclei. The changes in gene expression induced by experimental manipulations and the colocalization chemical messengers in hypothalamic neurosecretory neurons and its possible significance is summarized in this review.

Index Entries: Arcuate nucleus; medial preoptic area; paraventricular nucleus; supraoptic nucleus; pituitary; galanin; neurotensin; dynorphin; cholecystokinin; enkephalin; GABA; acetylcholine; neuropeptide Y; neuropeptide K; somatostatin; substance P; vasoactive intestinal polypeptide; peptide histidine-isoleucine; growth hormone-releasing factor; corticotropin-releasing factor; thyrotropin-releasing hormone; luteinizing hormone-releasing hormone; vasopressin; oxytocin; tyrosine hydroxylase; glutamate; aspartate; monosodium glutamate; neuropeptide; excitatory amino acid; transmitter; salt-loading; hypophysectomy; growth hormone; prolactin; adrenocorticotropin; neurosecretion; neuroendocrinology; colocalization; mRNA; *in situ* hybridization; immunohistochemistry.

Introduction

The neurosecretory cell represents an intermediate between endocrine cells and neurons, and the concept of neurosecretion refers to the release of a neurohormone into the blood stream from a nerve terminal. Neurosecretory cells were first discovered by Ernst and Berta Scharrer and Wilhelm Bargmann (Bargmann, 1949; Bargmann and Scharrer, 1951; Scharrer and Scharrer, 1954). They showed that the large (magnocellular) neurons in the paraventricular (PVN) and supraoptic (SON) nuclei produce neurosecretory products and transport them through the internal layer of the median eminence to the posterior lobe of the pituitary. Neurosecretory cells were later shown to be present not only in the magnocellular SON and PVN, but also in hypothalamic nuclei involved in control of anterior pituitary hormone secretion.

Hypothalamic neurosecretory neurons play a fundamental role in the brain control of endocrine organs and body homeostasis. Magnocellular neurosecretory neurons in the SON and PVN produce messenger molecules that are transported to the nerve terminals located in the posterior pituitary, where they are released into the systemic circulation. Parvocellular neurosecretory neurons produce the releasing or inhibiting hormones that are released into the portal circulation of the median eminence and control the secretion of the anterior pituitary hormones according to the concept of Harris (1955). The hypothalamic hormones have during the last decades been chemically characterized, primarily as peptides. In addition to the classical hypothalamic peptide hormones, including corticotropin-releasing factor (CRF), growth hormone-releasing factor (GRF), thyrotropin-releasing hormone (TRH), luteinizing hormone-releasing hormone (LHRH), and somatostatin (SOM), several other peptides have been demonstrated within hypothalamic neurons. Radioimmunological, immunohistochemical, and *in situ* hybridization techniques have defined levels, localization, and regulation of various peptides within hypothalamic regions. In many cases the additional peptides are encountered in the same neurons containing the classical hypophysiotropic factors. This is particularly evident in the magnocellular neurons of the SON and PVN and in the parvocellular neurons of the PVN and arcuate nucleus.

In the following chapter the chemical diversity and colocalization of various messenger molecules within neurosecretory neurons of hypothalamic nuclei will be reviewed. The state of activity of these neurons under "normal" and manipulated states has been studied with immunohistochemistry and *in situ* hybridization and some of these results will be presented. Functional aspects on the presence of additional messengers in hypothalamic neurons will also be considered.

Arcuate Nucleus

Chemical Messengers and Their Localization

The first compound to be chemically defined in cell bodies of the arcuate nucleus and in fibers of the median eminence was dopamine (Fuxe, 1964; Dahlström and Fuxe, 1964). Subsequent evidence showed that dopamine represents the major prolactin-inhibiting factor (PIF) (MacLeod and Lehmayer, 1974). For a long period dopamine was considered to be the only hypophysiotropic factor located in neurons of the arcuate nucleus.

However, almost two decades since the demonstration of dopamine in arcuate nucleus, GRF was demonstrated to have its location within cell bodies of the ventrolateral part of the arcuate nucleus (Sawchenko et al., 1985). During the last decade a large number classical transmitters and peptides have been discovered in the different parts of the arcuate nucleus, and for many of these substances direct or indirect effects on anterior pituitary hormone secretion have been demonstrated *(see* McCann, 1982).

The arcuate nucleus may anatomically be divided into three subdivisions; the dorsomedial part, the ventromedial part, and the ventrolateral part (Everitt et al., 1986; Meister and Hökfelt, 1988; Meister et al., 1989). The *dorsomedial* part contains the A12 dopamine neurons (Fuxe, 1964; Dahlström and Fuxe, 1964). The dopamine neurons, serving as the main prolactin regulating factor, contain in addition aminobutyric acid (GABA), and they produce two peptides, neurotensin (NT) and galanin (GAL) (*see* Everitt et al., 1986, Meister and Hökfelt, 1988). That these dorsomedial dopamine neurons are truly dopaminergic has been verified with the formaldehyde-induced fluorescence technique (Fuxe, 1964), and by demonstration of aromatic L-amino acid decarboxylase (AADC), the enzyme that converts L-DOPA into dopamine, as well as dopamine-immunoreactivity in these cells (Meister et al., 1988a; Okamura et al., 1988a,b). The *ventrolateral* part of the arcuate nucleus also contains a large number of tyrosine hydroxylase(TH)-containing cell bodies, however, they exhibit a less intense immunostaining compared to the dorsomedial ones, and they lack both AADC- and dopamine-like immunoreactivity (-LI) (Meister et al., 1988a; Okamura et al., 1988a,b). The dopaminergic nature of these cells is therefore uncertain, and it has been suggested that the ventrolateral TH-postive cells may release L-DOPA into the pericapillary space (*see* Meister et al., 1988a). L-DOPA may subsequently be converted into dopamine during its passage through the blood vessels of the portal plexus to the anterior pituitary. The true dopaminergic nature of the ventrolateral neurons may, however, in the future be revealed by demonstration of AADC mRNA in these cells. The TH-positive neurons in the ventrolateral arcuate nucleus also contain a large number of colocalized molecules, including GABA, choline acetyltransferase (ChAT; the acetylcholine-synthesizing enzyme), GRF, GAL, and NT (Everitt et al., 1986; Meister and Hökfelt, 1988) (Fig. 1). A smaller number of the TH-containing neurons also contain dynorphin (DYN), *leu*-enkephalin (ENK), and *met*-ENK-octapeptide (Everitt et al., 1986). In the ventrolateral part there are also propiomelanocortin (POMC)-containing neurons, however, they represent a separate set of neurons and have to date not been found to contain any other classical transmitter or peptide. Furthermore, the POMC-containing neurons do not give rise to fibers in the external layer of the median eminence, but project instead centrally.

In the *ventromedial* part of the arcuate nucleus, neuropeptide Y (NPY)- and SOM-containing neurons are present. The NPY-containing neurons are mainly confined to the ventral aspect of this subdivision of the nucleus, whereas the SOM-containing neurons are found more dorsally, especially in the caudal part of the nucleus. Single neurons have been reported to contain both peptides (Chronwall et al., 1984), but the majority of the SOM- and NPY-positive neurons have so far not been reported to contain additional messengers *(see also below).* Apart from the abovementioned peptides, there are neurons that are less clearly organized within the three different parts of the nucleus, often between the compartments and/or extending into several of them. This is the case for several ENK-, DYN, and substance P/neuropeptide K(NPK)-immunoreactive neurons.

Monosodium Glutamate-Induced Lesion of the Arcuate Nucleus

When the monosodium salt of glutamate (monosodium glutamate; MSG) is administered parenterally (1–4 mg/g body weight) during the first 2 wk after birth, a specific lesion is induced in the arcuate nucleus as first described by Olney (1969). The MSG-induced lesion has in a large number of studies been used in order to charac-

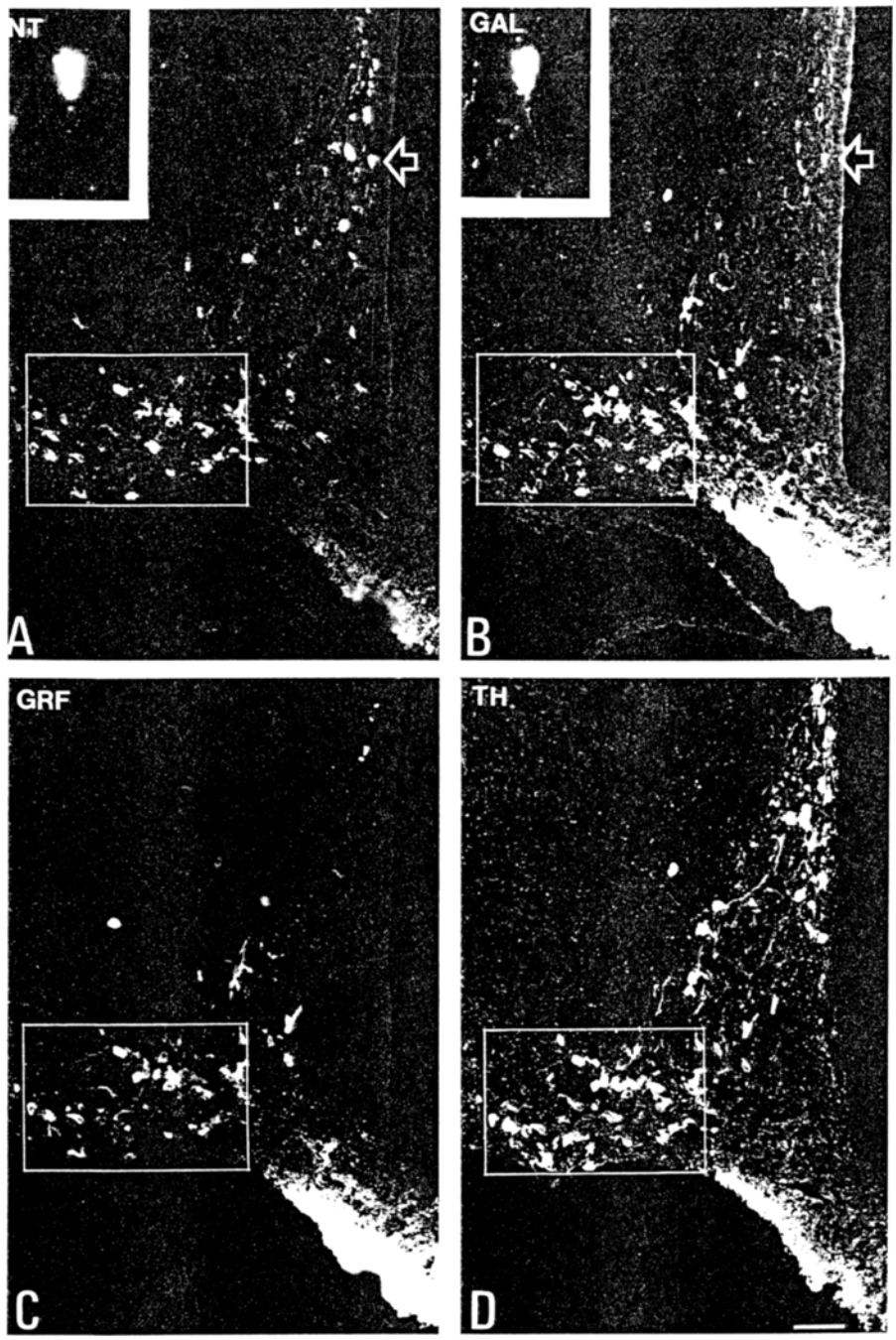
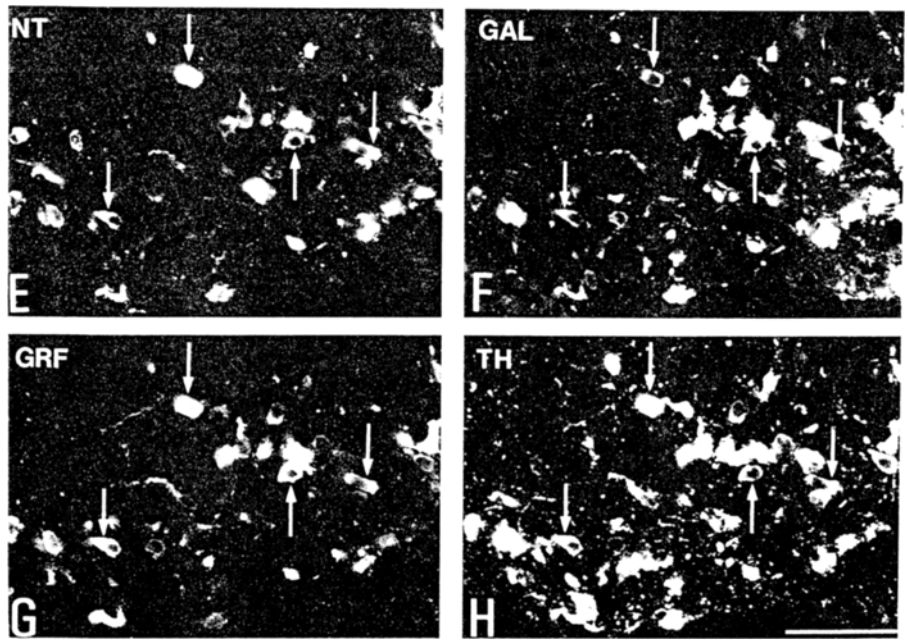


Fig 1. Immunofluorescence photomicrographs of the same section of the arcuate nucleus processed with two subsequent double-labeling techniques using antisera to neurotensin (NT) (**A**,**E**), galanin (GAL) (**B**,**F**), growth hormone-releasing factor (GRF) (**C**,**G**), and tyrosine hydroxylase (TH) (**D**,**H**). Rectangles in **A-D** indicate higher magnifications shown in **E–H**. In **A** and **B**, double-labeling with mouse monoclonal antibodies to NT and rabbit polyclonal antiserum to GAL was performed. After photography, the section was eluted with acid potassium permanganate ($KMnO_4$) in order to eliminate the previous staining. The section was subsequently reincubated with rabbit antiserum to GRF (**C**) and thereafter eluted again and reincubated with *(Continued on next page)*



(Continued from previous page) rabbit antiserum to TH (**D**). It can be seen that the vast majority of the neurons in the ventrolateral part of the arcuate nucleus contain NT, GAL, GRF, and TH *(see* arrows in **E-H**). In the dorsomedial part of the nucleus a neuron containing both NT and GAL is observed *(see* open arrow in **A** and **B**). Bars = 50 μm. (Modified from Meister and Hökfelt, 1988).

terize the chemical neuroanatomy and neuroendocrine role of the arcuate nucleus and projections to the median eminence *(see* Meister, 1991).

MSG is toxic to cell bodies possessing glutamate receptors within the arcuate nucleus. Glial cells or axons passing through the nucleus, lacking the amino acid receptor, are spared by the neurotoxic action. About 80–90% of the cell bodies in the arcuate nucleus appear to be susceptible to the effects of MSG. The lesion is restricted to the ventrolateral, ventromedial, and to a lesser extent to the dorsomedial part of the arcuate nucleus. The remaining dorsomedial cell group is condensed and ventrally dislocated to the ventrolateral corner of the third ventricle. Parenteral adminstration of MSG causes well characterized endocrine, metabolic, and behavioral abnormalities, including mainly stunted skeletal growth, severe obesity, and tail autoingestion. Histochemically, MSG causes a complete loss of cell bodies containing GRF, GAL, DYN, ENK, NPY, POMC, and NPK *(see* Fig. 2). Cells containing TH, GAD, NT, and SOM are always detected in the ventrally dislocated dorsomedial division of the arcuate nucleus. These changes are combined with marked decreases in numbers of fibers of the median eminence containing GRF, GAL, GAD, DYN, and ENK, suggesting that the arcuate cell bodies containing the above mentioned immunoreactivities contribute to the fiber network in the median eminence (Fig. 2a–d). Fibers containing TH, SOM, and NPY are, however, not affected by MSG (Fig. 2e–h). These observations suggest that the TH-immunoreactive fiber plexus in the median eminence originates from the remaining ventrolaterally dislocated cell group, that SOM-containing fibers in the median eminence originate from the periventricular area, and that NPY-containing fibers in the external layer of the median eminence have an origin in the lower brainstem, since they also contain dopamine β-hydroxylase *(see* Meister et al., 1989). The histochemistry of the arcuate nucleus and the projections to the median eminence are schematically summarized in Fig. 3.

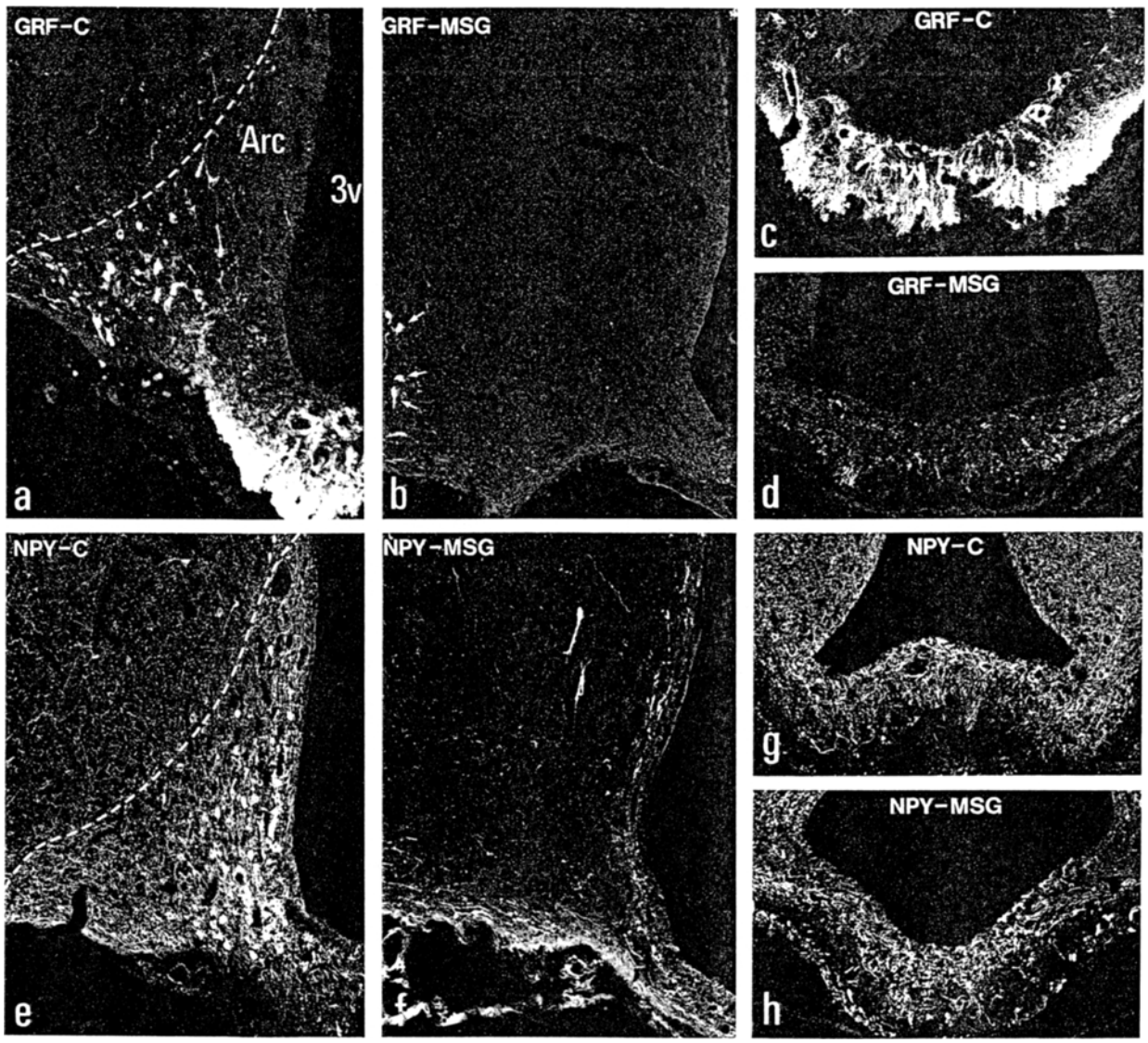


Fig. 2. Immunofluorescence photomicrographs of sections of the arcuate nucleus from control (C) (**a**,**c**,**e**,**g**) and MSG-treated (**b**,**d**,**f**,**h**) rats after incubation with antiserum to growth hormone-releasing factor (GRF) (**a**–**d**) and neuropeptide Y (NPY) (**e**–**h**). In control rats, GRF-immunoreactive (IR) cell bodies are located in the ventrolateral part of the arcuate nucleus (Arc; outlined with broken line) (**a**), whereas NPY-IR cell bodies are distributed in the ventromedial part of the nucleus (**e**). After MSG treatment, a complete loss of GRF- and NPY-containing cell bodies is seen in the arcuate nucleus (**b**,**f**). Some remaining GRF-IR cells are seen lateral to the ventromedial nucleus (small arrows in **b**), and some NPY-IR neurons are seen dorsal to the remaining arcuate nucleus (**f**). In the median eminence of control rats, a very dense plexus of GRF-immunoreactive (IR) fibers is seen in the external layer (**c**), whereas NPY-IR fibers are mainly seen in the internal layer (**g**). After MSG treatment, there is a marked reduction in GRF-IR fibers (**d**), indicating that GRF-containing cell bodies in the ventrolateral part of the arcuate nucleus project to the median eminence. No change in numbers of NPY-IR fibers is observed after MSG administration (**h**), suggesting that the NPY-containing cell bodies in the ventromedial part of the arcuate nucleus do not send projections to the median eminence. (Modified from Meister et al., 1989).

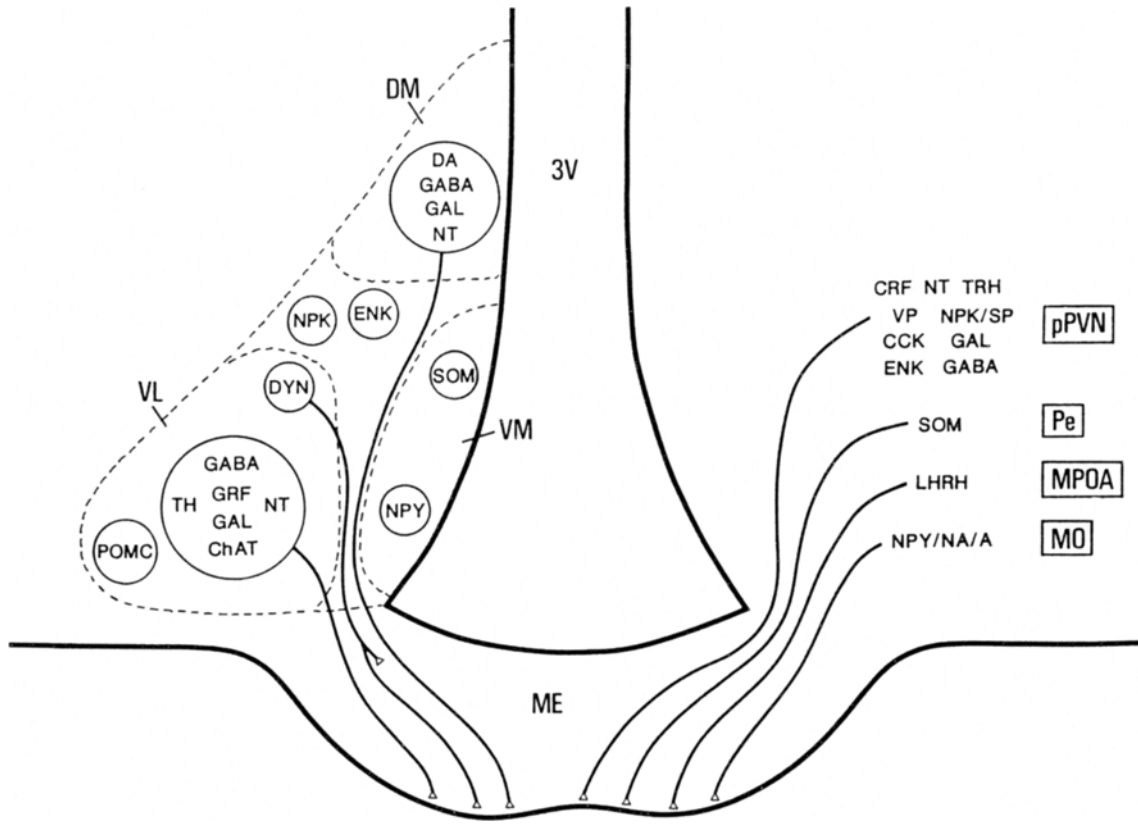


Fig. 3. Schematic drawing illustrating the distribution of neuroactive compounds in different parts of the arcuate nucleus and afferents to the median eminence as revealed with MSG-neurotoxicity. Anatomical abbreviations: DM = dorsomedial; MO = medulla oblongata; MPOA = medial preoptic area; Pe = periventricular nucleus; pPVN = parvocellular paraventricular nucleus; VM = ventromedial; VL = ventrolateral; and 3V = third ventricle. Abbreviations for transmitters and peptides: A = adrenaline; CCK = cholecystokinin; ChAT = choline acetyltransferase; CRF = corticotropin-releasing factor; DA = dopamine; DYN = dynorphin; ENK = enkephalin; GABA = gamma-aminobutyric acid; GAL = galanin; GRF = growth hormone-releasing factor, NA = noradrenaline; NPK = neuropeptide K; NPY = neuropeptide Y; NT = neurotensin; POMC = proopiomelanocortin; SOM = somatostatin; SP = substance P; TI = tyrosine hydroxylase; TRH = thyroptropin-releasing hormone; and VP = vasopressin. (From Meister et al., 1989).

Functional Aspects

The two major hypophysiotropic factors located in the arcuate nucleus are dopamine and GRF, located in the dorsomedial and ventrolateral part of the arcuate nucleus, respectively. It seems subsequently appropriate to assume that the two main functions of cell bodies in the the arcuate nucleus are to regulate prolactin and growth hormone (GH) secretion and that the additional comessengers may have synergistic effects on these main actions. In fact, it is well established that GABA, to a large extent colocalized with dopamine, also has an inhibitory effect on prolactin secretion (*see* Racagni et al., 1982; McCann and Rettori, 1986; *see also* Meister and Hökfelt, 1988), in agreement with its colocalization with dopamine in dorsomedial arcuate neurons. The GRF neurons contain a large number of putative messenger molecules, of which GAL is of special interest. The peptide GAL has been shown to stimulate GH secretion when given intraven-

tricularly (Melander et al., 1987; Ottlecz et al., 1986,1988; Murakami et al., 1987) and parenterally in both rats (Cella et al., 1988; Murakami et al., 1989; Hulting et al., 1991) and humans (Bauer et al., 1986; Davis et al., 1987; Hulting et al., 1991). The GH releasing effect induced by GAL seems, however, not to be excreted directly at the pituitary level, since there are no binding sites for GAL in the anterior pituitary (Gaymann and Falke, 1990; Hulting et al., 1991). Moreover, GAL does not appear to have any effect on cultured pituitary cells (Ottlecz et al., 1986,1988; Meister and Hulting, 1987) and the GAL-induced GH secretion in both rat and human is seen with a temporal delay (Hulting et al., 1991). More recently it has been shown that GAL in vitro releases GRF from fragments of the hypothalamus (Kitajima et al., 1990). The majority of GRF neurons in the monkey infundibular nucleus and median eminence also contain GAL (Meister et al., 1990a), indicating that GRF/GAL colocalization is not only confined to rats. Thus, evidence suggests that GAL is present with GRF in ventrolateral neurons of the arcuate nucleus and that GAL exhibits synergistic effects with GRF to release GH, most probably via a hypothalamic action to release GRF. The role of the other coexisting compounds with GRF is more unclear, but it is of interest that acetylcholine promotes GH secretion, both directly at the pituitary level (*see* Quabbe, 1986) and at the hypothalamic level via inhibition of SOM release (Locatelli et al., 1986).

Anterior Hypothalamic Regions

LHRH Neurons in the MPOA and OVLT

The LHRH containing neurons in the medial preoptic area (MPOA) and organum vasculosum of lamina terminalis (OVLT) have so far only been found to contain a very limited amount of cotransmitters. Recently the peptide GAL was demonstrated in a subset of OVLT and preoptic LHRH neurons (Merchenthaler et al., 1990), and functional studies have shown that GAL enhances in vitro release of LHRH from nerve terminals in the median eminence (Merchenthaler et al., 1990). The distribution of neurons coexpressing GAL and LHRH exhibit a sexual difference. In male rats about 20% of LHRH neurons are also immunopositive for GAL, whereas in female rats, about 65% of LHRH-containing perikarya contain GAL-LI (Merchenthaler et al., 1991). Female rats have higher levels of GAL in the preoptic area and median eminence in proestrous as compared to estrous, suggesting that gonadal steroids, primarily estrogen, may physiologically regulate the expression of GAL in a subpopulation of LHRH neurons (*see* Merchenthaler et al., 1991). Interestingly, estrogen has previously been reported to represent an important regulator of GAL gene expression in both the pituitary (Kaplan et al., 1988; Vrontakis et al., 1989) and brain (Gabriel et al., 1990). In the pituitary, estrogen has been found to cause at least a 1000-fold increase in GAL expression (Kaplan et al., 1988; Vrontakis et al., 1989). Furthermore, a sexual difference in GAL-like immunoreactivity (LI) in rat hypothalamus has been demonstrated using RIA measurements (Gabriel et al., 1989), showing that during sexual maturation the concentrations of GAL-LI increase in the pituitary and median eminence of the female rat.

Somatostatin Neurons in the Periventricular Nucleus

The somatostatin-containing neurons of the anterior hypothalamic periventricular nucleus give rise to the extensive fiber network in the external zone of the median eminence, whereas the somatostatin neurons in the the arcuate nucleus do not seem to give any major contribution to this fiber network (*see* Meister et al., 1989). In spite of the high number of somatostatin neurons surrounding the third ventricle in the anterior periventricular area, no other messenger molecule has been demonstrated in these somatostatin neurons. In other areas, such as the cerebral cortex, somatostatin colocalizes with GABA and NPY (*see* Meister and Hökfelt, 1992).

Parvocellular Paraventricular Nucleus

The parvocellular part of the paraventricular nucleus contains two main hypophysiotropic hormones; corticotropin-releasing factor (CRF) and thyrotropin-releasing hormone (TRH). Apart from these two peptides, immunohistochemical evidence has accumulated showing that a large number of additional peptides and one classical transmitter (GABA) are present in parvocellular PVN neurons. Since the discovery of CRF in the early 1980s, the exact location and projections of CRF-containing neurons have been clarified. It was shown early that some of the parvocellular CRF neurons also express vasopressin after adrenalectomy (Tramu et al., 1983; Kiss et al., 1984; Sawchenko et al., 1984; *see* Swanson et al., 1986), which is of particular interest, since vasopressin is known to be a secretagogue for adrenocorticotropin (ACTH), and thus acts synergistically with CRF. The CRF neurons exhibit an extensive coexistence with several other peptides. They have been shown to contain NT and ENK in larger proportions, whereas smaller numbers of CRF neurons contain GAL, cholecystokinin (CCK), and peptide histidine-isoleucine (PHI)/vasoactive intestinal polypeptide (VIP) (Ceccatelli et al., 1989). The CRF neurons have so far been shown to contain one classical transmitter, namely GABA. In the dorsomedial part of the parvocellular PVN, several CRF neurons contain GAD- and GABA-immunoreactivity (Fig. 4), and a substantial number of the GAD/CRF-containing cells have been suggested to project to the median eminence as revealed with retrograde tracing (Meister et al., 1988b). As would be expected, TRH- and CRF-containing neurons represent separate cell populations, and surprisingly the TRH-containing neurons have been found to contain a very limited number of additional messengers.

Although the role for CRF is well established, the functional significance of NT, ENK, GAL, VIP/PHI, and GABA in these neurons is more unclear. It has, however, been found that NT (Rivier et al., 1977; Maeda and Frohman, 1978), Vijayan and McCann, 1979) and VIP/PHI (Kato et al., 1978; Ruberg et al., 1978) have stimulatory effects on

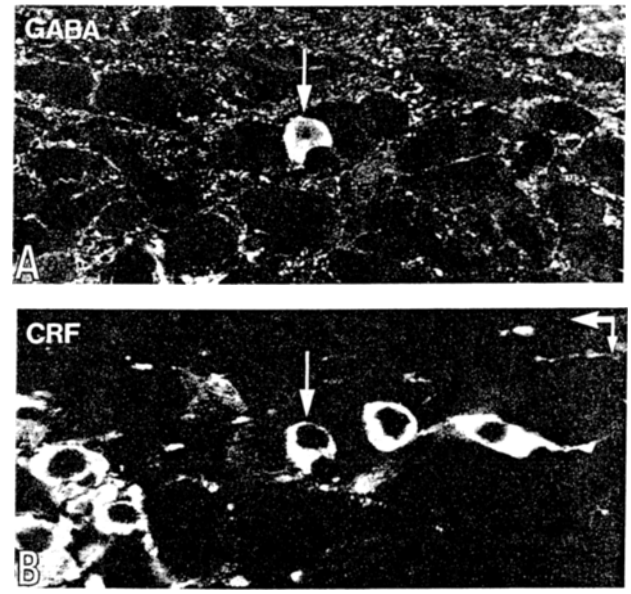


Fig 4. Immunofluorescence photomicrographs of a section of the parvocellular PVN after incubation with antiserum to GABA (**A**) and subsequent elution and restaining with antiserum to rat CRF (**B**). Comparison of **A** with **B** shows that a GABA cell in the dorsal aspect of the nucleus also contains immunoreactivity for CRF (*see* arrow). Note also distribution of a dense GABA-immunoreactive fiber plexus in the PVN, where the fibers closely surround CRF cells. Large arrow points in a medial direction and small arrow in ventral direction. (From Meister et al., 1988b).

prolactin secretion, and that prolactin is released during stress. Colocalization of NT and VIP/PHI with CRF may support the idea that NT and VIP/PHI could mediate this stress-induced release of prolactin parallel to the release of ACTH. Several studies indicate that GABA is also of importance in the control of ACTH secretion, mainly to inhibit the release of ACTH by a hypothalamic action, most likely via CRF (McCann and Rettori, 1986; Meister et al., 1988b).

Magnocellular Paraventricular and Supraoptic Nuclei

The two main products of the magnocellular hypothalamic neurons were early shown to be vasopressin (Du Vigneaud et al., 1953a), a hormone with main actions to maintain arteriolar

perfusion pressure and extracellular fluid balance, and oxytocin (Du Vigneaud et al., 1953b), involved in smooth muscle contractability. Besides these well known actions, other roles for vasopressin and oxytocin have been described, and both nonapeptides have been reported to be present in several peripheral tissues. Apart from the main neurohypophysial hormones, the magnocellular neurons transcribe, translate, and store several other neuroactive substances (*see* Brownstein and Mezey, 1986).

The placticity of the magnocellular system may easily be studied after experimental manipulations. In the following the changes in gene expression and changes in peptide/protein levels after two such manipulations, salt-loading and hypophysectomy, will be presented. The results suggest that in some cases the colocalized peptides are expressed in a different way as compared to the classical magnocellular hormones.

Hyperosmolarity-Induced Changes in Gene Expression

Administration of hyperosmotic stimuli, usually carried out by replacing tap water by 2% saline, induces several changes in the neurohypophysial system. These include increase in cell size (Kalimo, 1975), decreased glial contacts in the SON (Hatton et al., 1984), decrease in pituitary vasopressin and oxytocin levels (Zerbe and Palkovits, 1984) and increased gene expression for vasopressin (Sherman et al., 1986a,b,1988; Meister et al., 1990b), oxytocin (Van Tol et al., 1987; Meister et al., 1990b), TH (Young et al., 1987; Meister et al., 1990b; Watts, 1992), GAL (Rökaeus et al., 1988; Meister et al., 1990b, Young et al., 1990), DYN (Sherman et al., 1988; Meister et al., 1990b), CCK (Sherman et al., 1988; Meister et al., 1990b; Watts, 1992), VIP/PHI (Watts, 1992), and CRF (Young, 1986; Young et al., 1986; Watts, 1992).

The changes in gene expression for several of the substances colocalized in vasopressin and oxytocin neurons have been analyzed and correlated with changes in the immunoreactive peptide/protein products. This comparison may reflect parallel events in synthesis, transport, and secretion (Meister et al., 1990b). After prolonged salt-loading, increased levels of mRNA were demonstrated in the SON/PVN for vasopressin, oxytocin, TH, GAL (Fig. 5), and DYN. A substantial increase in CCK mRNA was also observed in the magnocellular PVN, whereas the increase in the SON was moderate. In noncolchicin treated/salt-loaded rats, a marked increase in TH-LI in cell bodies of the SON/PVN was evident, whereas vasopressin- and oxytocin-LI showed marked decreases, reaching almost nondetectable levels. In cell bodies of colchicine-treated/salt-loaded animals, decreases in GAL- (Fig. 5) and DYN-LI, but no certain changes in CCK-LI could be demonstrated. At the level of the posterior pituitary in noncolchicine-treated animals, similarly a decrease for vasopressin-, oxytocin-, GAL (Fig. 5), and DYN-LI were shown. However, there was no difference in numbers of TH-IR fibers, in agreement with the idea that TH is a cytoplasmic nonreleasable enzyme, and there was no demonstrable change in numbers of CCK-IR fibers. The abovementioned findings suggest that there is hyperosmolarity-induced synthesis of both vasopressin and oxytocin as well as their colocalized compounds GAL, DYN, and CCK. Since a depletion of immunoreactive peptide was seen at the level of the posterior pituitary and within the cell bodies of the SON/PVN, it may be concluded that the rate of transport and secretion exceeds the rate of synthesis (Fig. 6). These observations are in agreement with the demonstration that in osmotically stressed rats biosynthesis and axonal transport of hormone/peptides is augmented about fivefold (Gainer et al., 1977; Brownstein et al., 1980). In addition to the increase in biosynthetic rate, the prohormones are posttranslationally processed at a threefold more rapid rate (*see* Castel et al., 1984). These changes are consistent with the need for maintaining an augmented secretion rate under conditions of hormone depletion. In spite of the increased synthesis in magnocellular neurons observed after salt-loading, colchicine does not seem to be sufficient enough to build up high levels of immunoreactive material. This may be explained by the increased axonal transport and by the fact that colchicine treatment of salt-loaded rats inhibits transport less well than in normal rats (Peña et al., 1988).

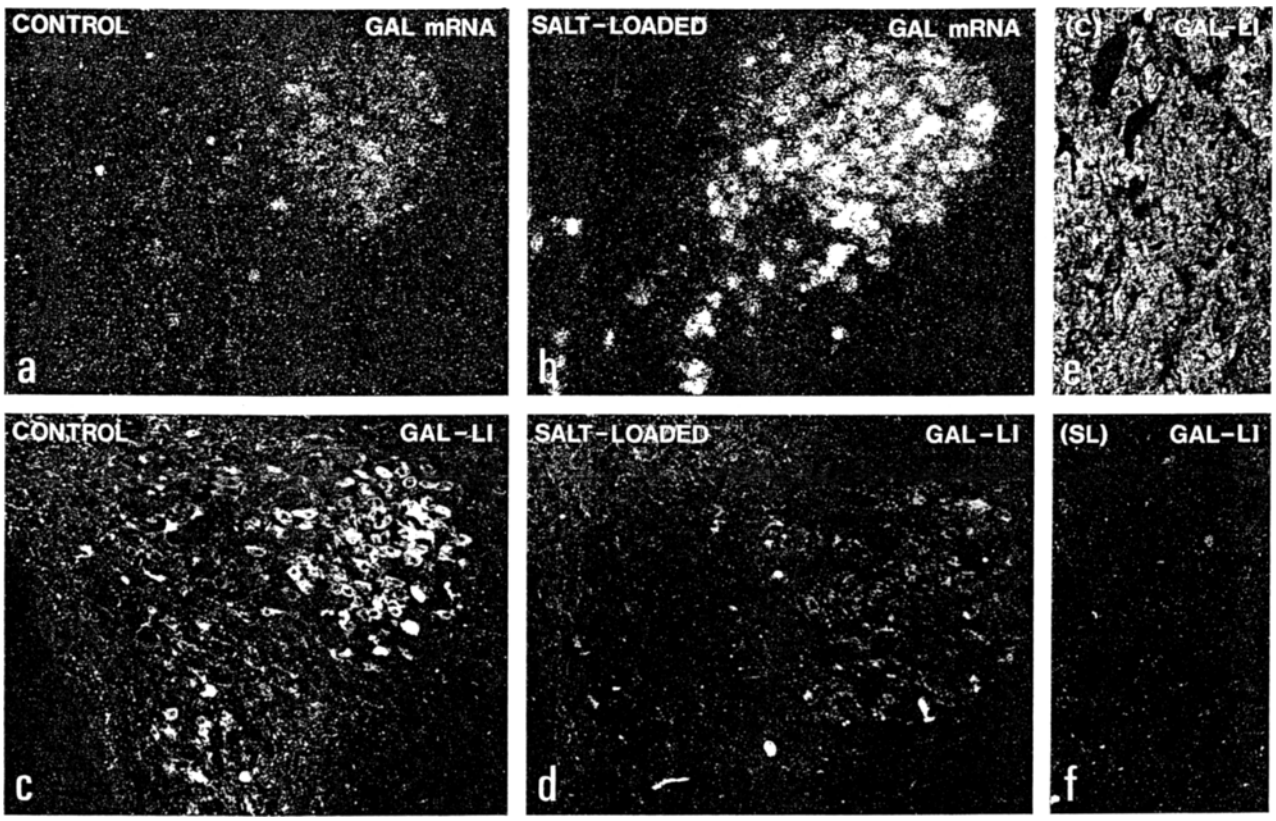


Fig. 5. Photomicrographs of emulsion-dipped (**a,b**) and immunofluorescence (**c–f**) sections of the paraventricular nucleus (PVN) (**a–d**) and posterior lobe of the pituitary (**e,f**) illustrating galanin (GAL) mRNA (**a,b**) and GAL-like immunoreactivity (-LI) in control (**a,c,e**) and salt-loaded (**b,d,f**) rats. In **c** and **d**, colchicine was adminstered 24 h before sacrifice. After salt-loading, there is an increase in GAL mRNA (cf. **a** with **b**) and a depletion of GAL-LI within the cell bodies of the PVN (cf. **c** with **d**) as well as in nerve fibers of the posterior pituitary (cf. **e** with **f**). The figure should be compared with schematic drawing in Fig. 6. (Modified from Meister et al., 1990b).

However, other mechanisms should also be considered, including changes in processing of the precursor and the formation of new products that may not be recognized by the antisera used in immunohistochemical analysis.

Effects of Hypophysectomy/Nerve Transection on Gene Expression

Removal of the pituitary gland, i.e., hypophysectomy, includes two main alterations to the experimental animal, lack of pituitary hormones and transection of the nerve axons from the SON and PVN to the posterior pituitary. The expression of neurohypophysial hormones/peptides has been studied with combined *in situ* hybridization and immunohistochemistry (Villar et al., 1990). Five days after hypophysectomy a dramatic increase in GAL (Figs. 7,8) and CCK mRNA and GAL- and CCK-immunoreactivity was demonstrated in the SON and magnocellular PVN. In addition, some parvocellular CCK neurons in the PVN could be demonstrated. DYN, however, showed a differential change after hypophysectomy, with a decrease in DYN mRNA and increase in DYN-immunoreactivity (Fig. 7). Only smaller decreases in mRNA and immunoreactivity were observed for vasopressin and oxytocin. There were also temporal changes in mRNA during the different

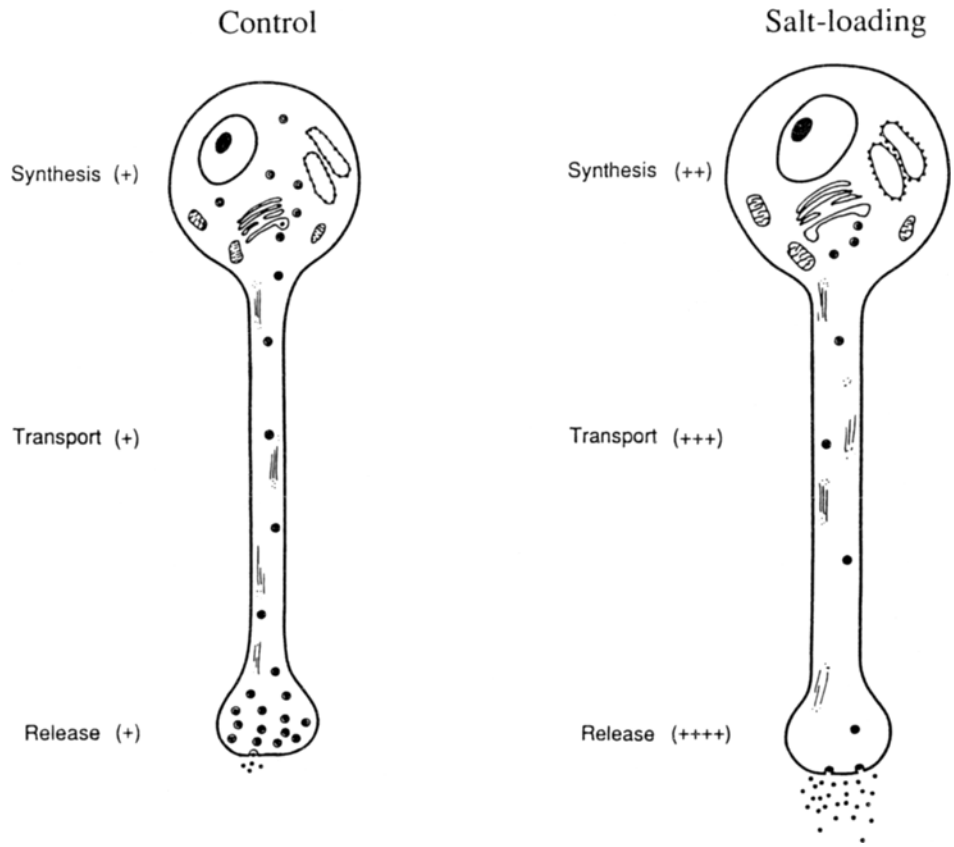


Fig. 6. Schematic drawing of a magnocellular neuron of a control and salt-loaded rat. In the control neuron, synthesis, transport, and release occur at a similar rate. In the salt-loaded neuron, there is an increase in synthesis, transport, and release, where the rate of release exceeds the rate of transport and synthesis, leading to a depletion of immunoreactive peptide at the nerve terminal and cell body level. Note hypertrophy of the salt-loaded neuron. *See* Fig. 5 and text for further information.

survival times with an increase in GAL mRNA as early as 2 d after hypophysectomy, reaching a maximum at 5 d and thereafter gradually declining during 7 and 14 d to reach normal values at 36 d (Fig. 8). Similarly, CCK levels reached peak values at around 5 d posthypophysectomy and thereafter decreased. That CCK mRNA in addition showed an increase in the parvocellular neurons of the PVN suggests that the removal of the anterior pituitary induces increased CCK mRNA levels in this part of the nucleus. The results from this study have shown that the transient expression of several peptides in magnocellular neurons is altered in differential ways after hypophysectomy. The changes observed in expression of peptides and their mRNAs in hypophysectomized animals should be considered against the well known fact that transection of the neurohypophysial tract causes extensive degeneration in magnocellular neurons of the SON and PVN. Of particular interest is that the peptides GAL and CCK markedly increase after hypophysectomy. Upregulation of GAL peptide and GAL mRNA has been reported in dorsal root ganglia after transection of the sciatic nerve (Hökfelt et al., 1987; Villar et al., 1989), and lesions of the hippocampus results in increased GAL mRNA in the ipsilateral cholinergic forebrain neurons (Cortés et al., 1990). Taken together these observations suggest that GAL, and also CCK, may be peptides involved in degenerative and subsequent regenerative processes.

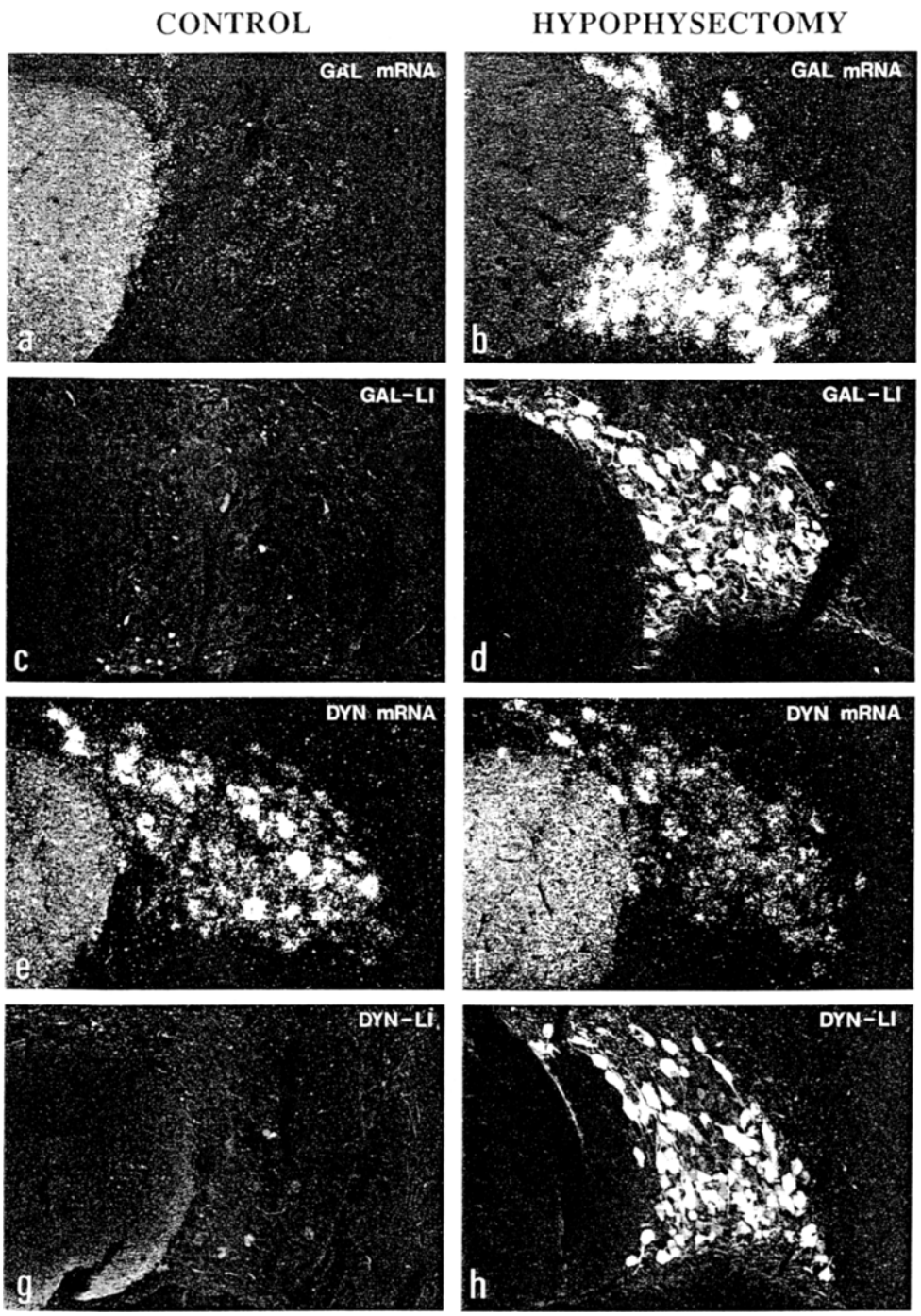


Fig. 7. Photomicrographs of emulsion-dipped (**a,b,e,f**) and immunofluorescence (**c,d,g,h**) sections of the supraoptic nucleus showing galanin (GAL) mRNA and GAL-like immunoreactivity (LI) as well as dynorphin (DYN) mRNA and DYN-LI in control (**a,c,e,g**) and hypophysectomized (**b,d,e,f**) rats (survival time 5 d). Hypophysectomy induces a dramatic increase in GAL mRNA (cf. **a** with **b**) and GAL-LI (cf. **c** with **d**). However, whereas there is an increase in DYN-LI (cf. **g** with **h**) after hypophysectomy, there is also a parallel decrease in DYN mRNA (cf. **e** with **f**), suggesting differential induction in GAL and DYN mRNAs after hypophysectomy. (Modified from Villar et al., 1990).

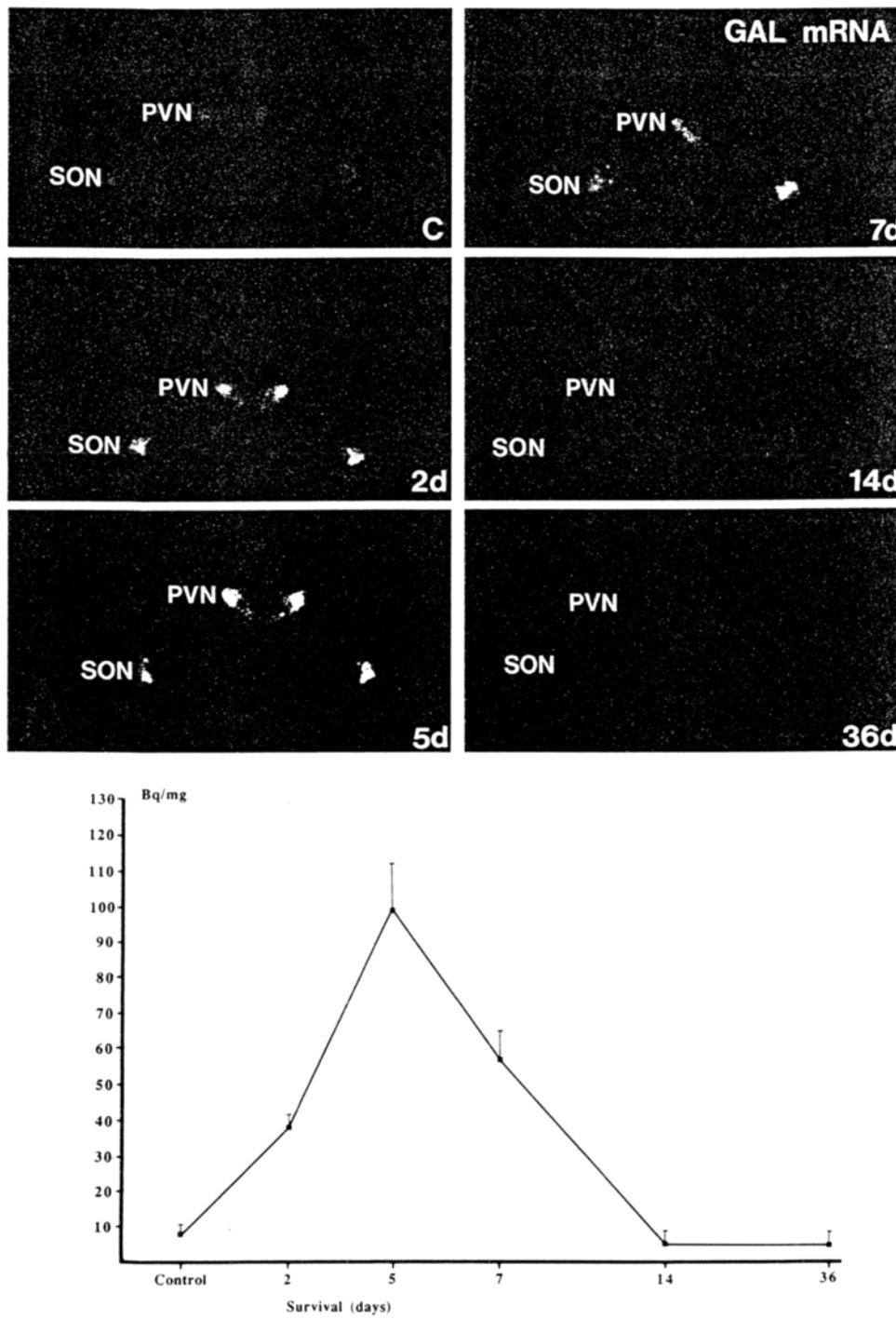


Fig. 8. Film autoradiograms showing expression of galanin (GAL) mRNA in magnocellular neurons of the SON and PVN during different survival times after hypophysectomy (**2,5,7,14,** and **36 d**). GAL gene expression in relation to time is depicted in the lower figure. In control (**C**) animals, low levels of GAL mRNA are expressed. Two days after hypophysectomy, there is an increase in GAL mRNA, which reaches its maximum at d 5, and thereafter declines at d 7. At d 14 and 36 posthypophysectomy, the levels of GAL mRNA have reached control values. (Modified from Villar et al., 1990).

Plasticity in Expression of Multiple Chemical Messengers

A large number of studies using immunocytochemistry and *in situ* hybridization have concluded that vasopressin and oxytocin are made in separate populations of magnocellular cells in normal animals (Aspeslagh et al., 1976; Rhodes et al., 1981; Hou-Yu et al., 1986; Mohr et al., 1988) (Fig. 9a,b). However, recent findings indicate that after certain experimental manipulations or physiological stimuli, such as lactation, many neurons of the SON can synthesize both hormones at the same time (Mezey and Kiss, 1991). Also, after salt-loading a smaller number of magnocellular neurons have been demonstrated to coexpress vasopressin and oxytocin mRNA (Kiyama and Emson, 1990).

A large number of additional messenger molecules are encountered in vasopressin and oxytocin neurons (*see* Brownstein and Mezey, 1986; Meister et al., 1990c). Many vasopressin neurons contain DYN (Gaymann and Martin, 1987; Watson et al., 1982; Whitnall et al., 1983; Meister et al., 1990c), *leu*-ENK (Martin and Voigt, 1981; Martin et al., 1983; Gaymann and Martin, 1987), GAL (Brownstein and Mezey, 1986; Rökaeus et al., 1988; Gaymann and Martin, 1989; Meister et al., 1990c) (Fig. 9c,d), and TH (Meister et al., 1990c), whereas oxytocin neurons contain cholecystokinin (CCK) (Vanderhaeghen et al., 1981; Martin et al., 1983; Meister et al., 1990), CRF (Burlet et al., 1983; Sawchenko et al., 1984) (Fig. 9e,f), and met-ENK (Martin and Voigt, 1981; Martin et al., 1983; Vanderhaeghen et al., 1983; Adachi et al., 1985; Gaymann and Martin, 1987) and TRH (Tsuruo et al., 1988; Meister et al., 1990c) (*see* Fig. 10). Several of the mentioned combinations cannot be visualized after only colchicine-treatment, however, after hypophysectomy of salt-loading plus colchicine-treatment, colocalization can be demonstrated when the magnocellular neurons are under maximal synthetic capacity (Fig. 10). Thus, the expression of multiple chemical messengers in magnocellular neurons shows plasticity depending on the appropriate stimuli.

Functional Considerations

Although it has been evident for some years that the magnocellular neurons of the SON and PVN store a large number of other chemical messengers, the knowledge on the functional roles of these colocalized compounds in the neurohypophysis is fragmentary. It has been reported that dynorphin (mainly colocalized with vasopressin) inhibits the secretion of oxytocin (Bondy et al., 1988) and that CCK (mainly colocalized with oxytocin) stimulates both vasopressin and oxytocin secretion (Bondy et al., 1989a). Both effects can be blocked by naloxone and the CCK-antagonist L-364,718, respectively. In agreement, specific binding sites for DYN (Gerstberger and Barden, 1986; Herkenham et al., 1986) and CCK (Bondy et al., 1989a,b) have been demonstrated in the posterior pituitary. Both dehydration and salt-loading causes a parallel depletion of DYN and vasopressin (Lorens et al., 1985; Meister et al., 1990b), suggesting a concomitant release of both compounds from the posterior pituitary and a subsequent simultaneous inhibitory effect on neighboring oxytocin-containing terminals. The secretion of CCK also parallels the secretion of oxytocin (Sherman et al., 1988), which may suggest presence of presynaptic CCK receptors mediating further oxytocin release from the CCK/oxytocin-containing nerve terminal as well as postsynaptic CCK receptors that mediate stimulated release of vasopressin.

The peptide GAL, mainly present in vasopressin-containing nerve terminals, has so far not been shown to have any significant effect on either vasopressin of oxytocin secretion (Gaymann and Falke, 1990; Meister et al., 1993). However, a marked stimulatory effect on CCK release has recently been found (Meister et al., 1993). Dehydration and salt-loading, i.e., increase in plasma osmolality, which is known to deplete vasopressin and oxytocin from the neurohypophyseal system, cause a reduction in GAL-LI in cell bodies in the SON/PVN, in nerve fibers in the median eminence, and in nerve terminals in the neurohypophysis as demonstrated with RIA (Koenig et al., 1989; Skofitsch et al., 1989). Parallel to the decrease

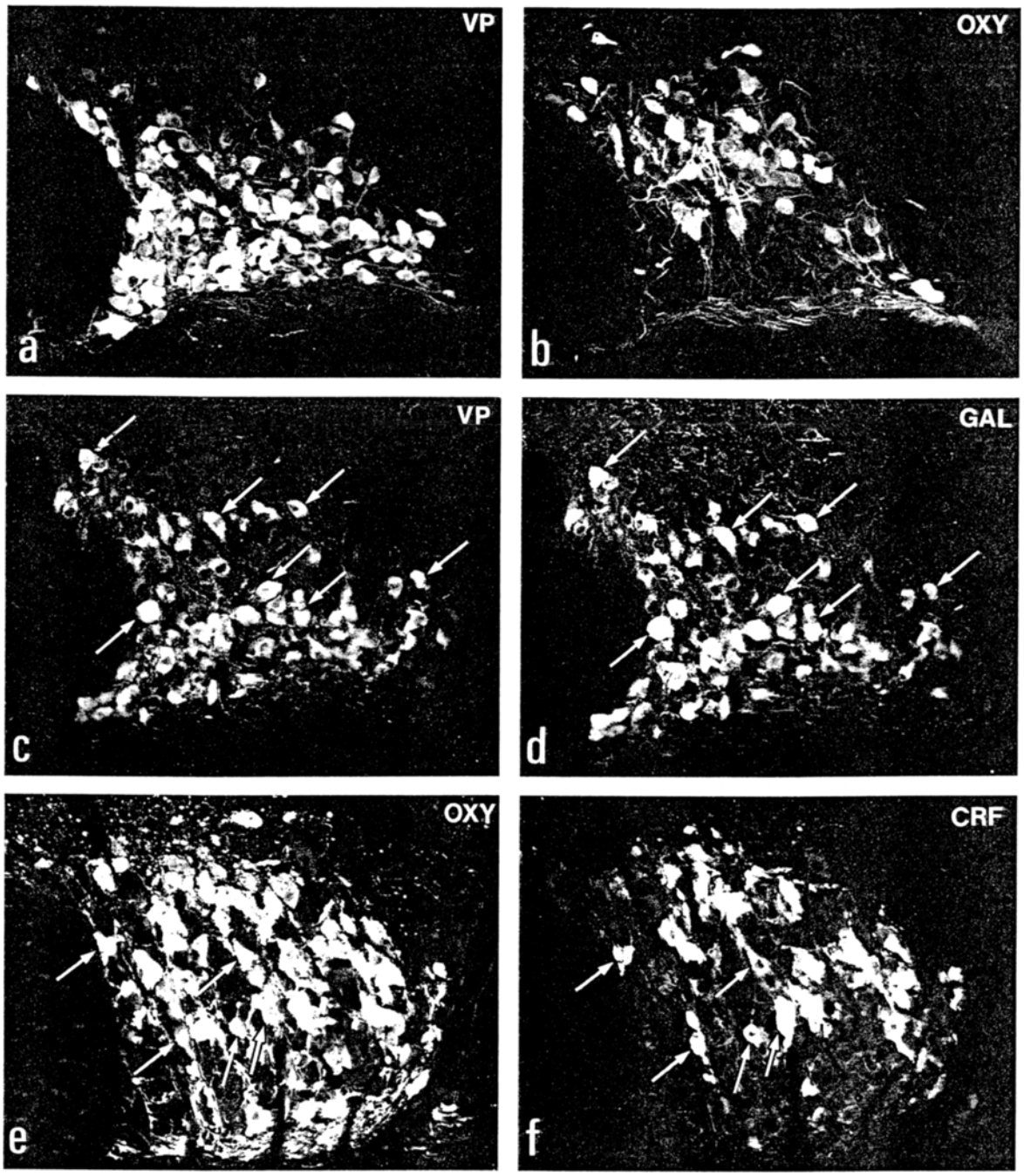


Fig. 9. Immunofluorescence photomicrographs of sections of the rat SON after double-staining with antisera to vasopressin (VP) (**a**) and oxytocin (OXY) (**b**), VP (**c**) and galanin (GAL) (**d**), and OXY (**e**) and corticotropin-releasing factor (CRF) (**f**) after hypophysectomy (**a–d**) and after salt-loading (**e,f**). It can be seen that in the hypophysectomized rat, VP and OXY-containing neurons represent separate cell populations (cf. **a** with **b**), whereas a large number of VP neurons also contain GAL (cf. **c** with **d**). In the salt-loaded rat, a large number of the OXY-containing cell population also contain CRF (cf. **e** with **f**). (Modified from Meister et al., 1990c).

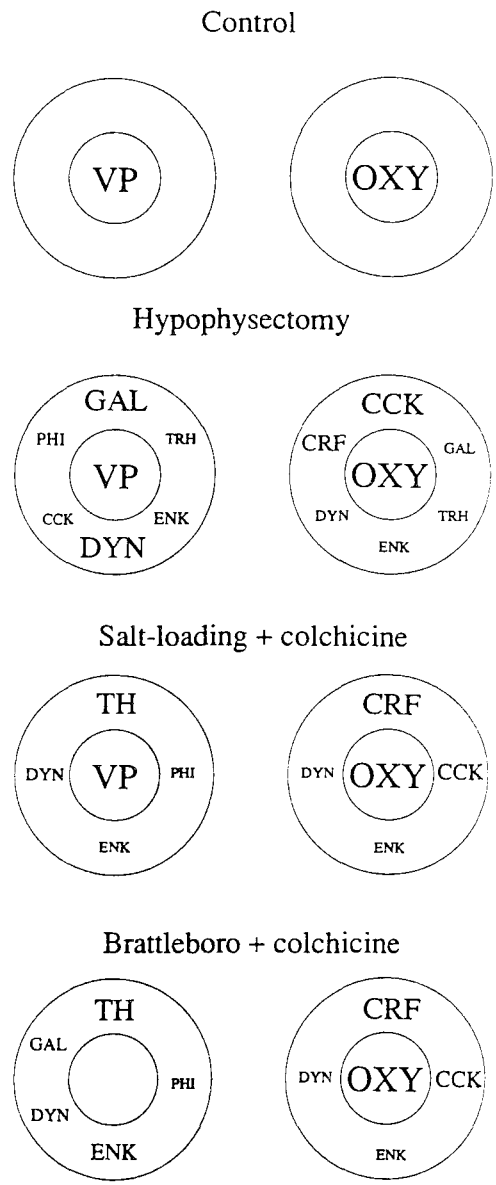


Fig. 10. Schematic drawing summarizing the proportional presence of additional chemical messengers in vasopressin (VP) and oxytocin (OXY) neurons after hypophysectomy, after salt-loading plus colchicine treatment and in the colchicine-treated Brattleboro rat. CCK = cholecystokinin; CRF = corticotropin-releasing factor; DYN = dynorphin; ENK = enkephalin; GAL = galanin; PHI = peptide histidine-isoleucine; SOM = somatostatin; TH = tyrosine hydroxylase; TRH = thyrotropin-releasing hormone. Note that the Brattleboro rat has a defect in the ability to synthesize VP. (From Meister et al., 1990c).

in GAL-LI, the levels of GAL mRNA in the SON and PVN increase during hyperosmolarity (Meister et al., 1990b; Young et al., 1990), indicating that the rate of transport through axons in the median eminence and the secretion from the neural lobe exceeds the rate of synthesis in the SON and PVN (Meister et al., 1990b) (*see* Fig. 6). When GAL is administered systemically there is a mild diuresis in rats, whereas GAL does not seem to influence the vasopressin-induced antidiuretic effect (Skofitsch et al., 1989).

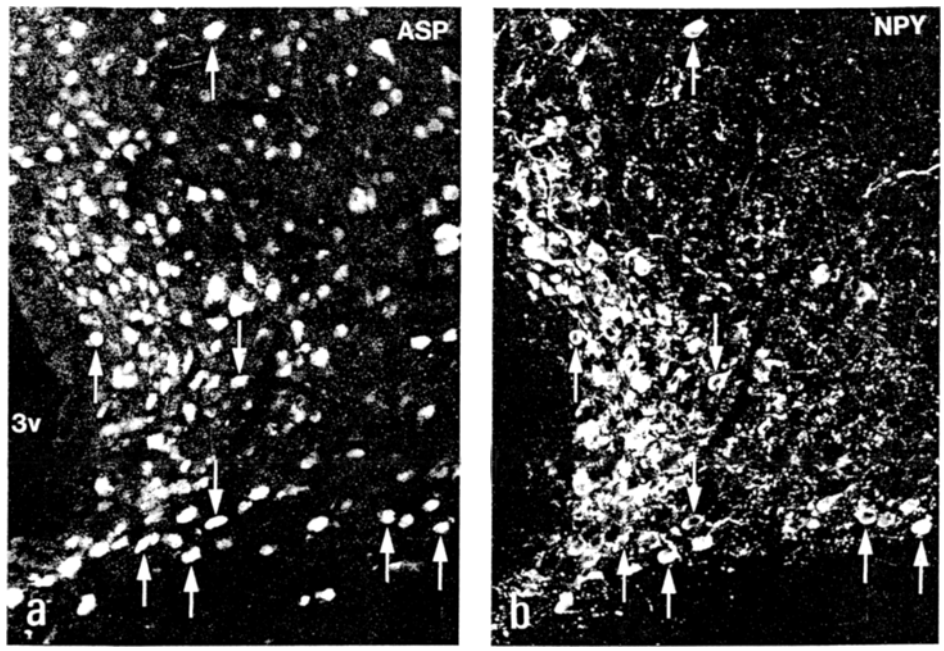


Fig. 11. Immunofluorescence photomicrographs of sections of the rat arcuate nucleus after double-staining with antisera to aspartate (ASP) (**a**) and neuropeptide Y (NPY) (**b**). In the ventromedial part of the arcuate nucleus, several ASP-positive neurons are distributed, and several of them also contain NPY-immunoreactivity (*see* arrows).

Excitatory Amino Acids in Neurosecretory Neurons

Recent studies have shown that the excitatory amino acids glutamate and asparate are widely distributed in the hypothalamus (Palkovits et al., 1986; Meeker et al., 1989; Van den Pol, 1990,1991). Particularly high concentrations of glutamate and aspartate are encountered in the hypothalamic magnocellular neurons (Palkovits et al., 1986), and it has been suggested that glutamate may be the main excitatory transmitter in neuroendocrine regulation (Van den Pol, 1990). Thus, magnocellular neurons of the SON respond to the application of glutamate by firing a train of stimulus bound action potentials (Arnauld et al., 1983; Biolac et al., 1978), and perifusion or iontophoretic application of glutamate to the magnocellular neurons elicits high frequency action potentials and bursting patterns that mimic the natural firing pattern of these cells in vivo (Haller and Wakerley, 1980). Specific antibodies to glutamate have shown that this excitatory amino acid is present in perikarya and dendrites of the magnocellular neurons of the SON as well as in terminal endings that synapse on these cells (Meeker et al., 1989). Recent experiments have given evidence for a widespread occurrence of glutamate and aspartate in neurosecretory neurons of the suprachiasmatic, periventricular, SON, PVN (both parvo- and magnocellular parts), and arcuate nuclei (Decavel and Van den Pol, 1992; Meister et al., in preparation). Within these nuclei, several neuropeptides have been demonstrated together with both glutamate and aspartate. For example, in the ventromedial part of the arcuate nucleus, aspartate is present in NPY-containing neurons (Fig. 11), and in the SON and magnocellular PVN, glutamate and aspartate are colocalized with both vasopressin and oxytocin.

Colocalization in hypothalamic neurosecretory neurons: current concepts

Area	Hormone	Colocalizing substance
- Medial preoptic area	LHRH	GAL
- Anterior periventricular nucleus	SOM	
- Arcuate nucleus	GRH Dopamine	TH, ACh, GABA, GAL, NT GABA, NT, GAL
- Paraventricular nucleus *(parvocellular part)*	CRH TRH	VP, NT, ENK, CCK, GAL, VIP/PHI, GABA
- Supraoptic nucleus Paraventricular nucleus *(magnocellular part)*	VP OXY	TH, GAL, DYN, PHI, ENK, TRH, CCK CRH, CCK, ENK, TRH, GAL

Fig 12. Summary of current concepts of colocalization in hypothalamic neurosecretory neurons. ACh = acetylcholine; CCK = cholecystokinin; CRH = corticotropin-releasing hormone; DYN = dynorphin; ENK = enkephalin; GABA = gamma-aminobutyric acid; GAL = galanin; GRH = growth hormone-releasing hormone; LHRH = luteinizing-releasing hormone; NT = neurotensin; OXY = oxytocin; PHI = peptide histidine-isoleucine; SOM = somatostatin; TH = tyrosine hydroxylase; TRH = thyrotropin-releasing hormone; VIP = vasoactive intestinal polypeptide; VP = vasopressin.

Summary and Conclusions

The majority of neurosecretory neurons in the hypothalamus have the genetic codes necessary to express more than one messenger molecule (Fig. 12). Within these neurosecretory neurons there is to date at least one hormone with a well characterized function in the regulation of anterior pituitary hormone secretion and in the regulation of physiological events in peripheral tissues. The function of messenger molecules colocalized with the classical hypothalamic hormone remains still rather unknown, but evidence suggests interactions between hypothalamic hormones and coreleased messenger molecules at the level of the median eminenceor at the level of the pituitary gland. A given stimuli may cause changes in gene expression for all colocalized compounds in neurosecretory neurons, but differential changes in gene expression also exist, suggesting diversity at the receptor level and in signal transduction pathways. Comparative analysis of changes in gene expression and changes in peptide/protein levels may provide information on rates of synthesis and secretion.

Acknowledgments

Supported by grants from the Swedish Medical Research Council (04X-10358), Tore Nilsons Fond, Magnus Bergvalls Stiftelse, Åhlén-Stiftelsen, and Sigurd and Elsa Goljes Stiftelse. I wish to thank Prof. Tomas Hökfelt for generous support.

References

Adachi T. Hisano S. and Daikoku S. (1985) Intragranular colocalization of immunoreactive methionine-enkephalin and oxytocin within the nerve terminals in the posterior pituitary. *J. Histochem. Cytochem.* **33**, 891–899.

Arnauld E., Cirino M., Layton B. S., and Renaud L. (1983) Contrasting actions of amino acids, acetylcholine, noradrenaline and leucine enkephalin on the excitability of supraoptic vasopressin-secreting neurons. *Neuroendocrinology* **36,** 187–196.

Aspeslagh M. R., Vandesande F., and Dierickx K. (1976) Electron microscopic immunocytochemical demonstration of separate neurophysin-vasopressinergic and neurophysioxytocinergic nerve fibers in the neural lobe of the rat hypophysis. *Cell Tissue Res.* **171,** 31–37.

Bargmann W. (1949) Über die neuroeskretorische Verknüpfung von Hypothalamus und Neurohypophyse. *Z. Zellforsch. Mikrosk. Anat.* **34,** 610–634.

Bargmann W. and Scharrer E. (1951) The site of origin of the hormones of the posterior pituitary. *Am. Sci.* **39,** 255–259.

Bauer F. E., Ginsberg L., Venetikou M., MacKay D. J., Burrin J. H., and Bloom S. R. (1986) Growth hormone release in man induced by galanin, a new hypothalamic peptide. *Lancet* **ii,** 192–194.

Bioloac B., Gafori O., Harris M., and Vincent J.-D. (1978) Effects of acetylcholine, sodium glutamate and GABA on the discharge of supraoptic neurons in the rat. *Brain Res.* **154,** 159–162.

Bondy C. A., Gainer H., and Russell J. T. (1988) Dynorphin A inhibits and naloxone increases the electrically stimulated release of oxytocin but not vasopressin from the terminals of the neural lobe. *Endocrinology* **122,**1321–1327.

Bondy C. A., Gainer H., Jensen R. T., and Brady L. A. (1989a) CCK evokes secretion of oxytocin and vasopressin from rat neural lobe independent of external calcium. *Proc. Natl. Acad. Sci. USA* **86,** 5198–5201.

Bondy C. A., Whitnall M. H., Brady L. S., and Gainer H. (1989b) Coexisting peptides in hypothalamic neuroendocrine systems: some functional implications. *Cell. Molec. Neurobiol.* **9,** 427–446.

Brownstein M. J. and Mezey E. (1986) Multiple chemical messengers in hypothalamic magnocellular neurons. *Progr. Brain Res.* **68,** 161–168.

Brownstein M. J., Russell J. T., and Gainer H. (1980) Synthesis, transport, and release of posterior pituitary hormones. *Science* **207,** 373–378.

Burlet A., Tonon M. C., Tankosic D., Coy D., and Vaudry H. (1983) Comparative immunocytochemical localization of corticotropin-releasing factor (CRF-41) and neurohypophyseal peptides in the brain of Brattleboro and Long-Evans rats. *Neuroendocrinology* **37,** 64–72.

Castel M., Gainer H., and Dellmann H. D. (1984) Neural secretory systems. *Int. Rev. Cytol.* **88,** 393–459.

Ceccatelli S., Eriksson M., and Hökfelt T. (1989) Distribution and coexistence of CRF-, neurotensin-, enkephalin-, cholecystokinin-, galanin- and VIP/PHI-like peptides in the parvocellular part of the paraventricular nucleus. *Neuroendocrinology* **49,** 309–323.

Cella S. G., Locatelli V., De Gennaro V., Bondiolotti G. P., Pintor C., Loche S., Provezza M., and Müller E. E. (1988) Epinephrine mediates the growth hormone-releasing effect of galanin in infant rats. *Endocrinology* **122,** 855–859.

Chronwall B. M., Chase T. N., and O'Donohue T. L. (1984) Coexistence of neuropeptide Y and somatostatin in rat and human cortical and rat hypothalamic neurons. *Neurosci. Lett.* **52,** 213–217.

Cortés R., Villar M. J., Verhofstad A., and Hökfelt T. (1990) Effects of central nervous system lesions on the expression of galanin: a comparative *in situ* hybridization and immunohistochemical study. *Proc. Natl. Acad. Sci. USA* **87,** 7742–7746.

Dahlström A. and Fuxe K. (1964) Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in cell bodies of brain stem neurons. *Acta Physiol. Scand.* **62,** Suppl. 232, 1–55.

Davis T. M. E., Burrin J. M., and Bloom S. R. (1987) Growth hormone (GH) release in response to GH-releasing hormone in man is 3-fold enhanced by galanin. *J. Clin. Endocrinol. Metab.* **65,** 1248–1252.

Decavel, C. and Van den Pol, A. N. (1992) Converging GABA- and glutamate-immunoreactive axons make synaptic contact with identified hypothalamic neurosecretory neurons. *J. Comp. Neurol.* **316,** 104–116.

Du Vigneaud V., Lawler H. C., and Popenoe E. A. (1953a) Enzymatic cleavage of glycinamide from vasopressin and a proposed structure for this pressor antidiuretic hormone of the posterior pituitary. *J. Am. Chem. Soc.* **75,** 4880–4881.

Du Vigneaud V., Ressler C., Swan J. M., Roberts C. W., Katsoyannis P. G., and Gordon S. (1953b) The synthesis of an octapeptide amide with hormonal activity of oxytocin. *J. Am. Chem. Soc.* **75,** 4878–4880.

Everitt B. J., Meister B., Hökfelt T., Melander T., Terenius L., Rökaeus Å., Theodorsson-Norheim E., Dockray G., Edwardson J., Cuello C., Elde R., Goldstein M., Hemmings H., Ouimet C., Walaas I., Greengard P., Vale W., Weber E. , Wu J. Y., and Chang K. J. (1986) The hypothalamic arcuate nucleus-median eminence complex: immunohistochemistry of transmitters, peptides and DARPP-32 with special reference to coexistence in dopamine neurons. *Brain Res. Rev.* **11,** 97–155.

Fuxe K. (1964) Cellular localization of monoamines in the median eminence and infundibular stem of some mammals. *Z. Zellforsch.* **61,** 719–724.

Gabriel S. M., Kaplan L. M., Martin J. B., and Koenig J. I. (1989) Tissue specific sex difference in galanin-like immunoreactivity and galanin mRNA during development in the rat. *Peptides* **10,** 369–374.

Gabriel S. M., Koenig J. I., and Kaplan L. M. (1990) Galanin-like immunoreactivity is influenced by estrogen in prepubertal and adult rats. *Neuroendocrinology* **51,** 168–173.

Gainer H., Sarne Y., and Brownstein M. J. (1977) Biosynthesis and axonal transport of rat neurohypophysial proteins and peptides. *J. Cell Biol.* **73,** 366–381.

Gaymann W. and Falke N. (1990) Galanin lacks binding sites in the porcine pituitary and has no detectable effect on oxytocin and vasopressin release from rat neurosecretory endings. *Neurosci. Lett.* **112,** 114–119.

Gaymann W. and Martin R. (1987) A re-examination of the localization of immunoreactive dynorphin (1–8), (Leu) enkephalin and (Met) enkephalin in the rat neurohypophysis. *Neuroscience* **20,** 1069–1080.

Gaymann W. and Martin R. (1989) Immunoreactive galanin-like material in magnocellular hypothalamo-neurohypophyseal neurons of the rat. *Cell Tissue Res.* **255,** 139–147.

Gerstberger R. and Barden N. (1986) Dynorphin 1–8 binds to opiate kappa receptors in the neurohypophysis. *Neuroendocrinology* **42,** 376–382.

Haller E. W. and Wakerley J. B. (1980) Electrophysiological studies of paraventricular and supraoptic neurons recorded in vitro from slices of rat hypothalamus. *J. Physiol. Lond.* **302,** 347–362.

Harris G. (1955) *Neural Control of the Pitutary Gland.* Edward Arnold, London.

Hatton G. I., Perlmutter L. S., Salm A. K., and Tweedle C. D. (1984) Dynamic neuronal-glial interactions in hypothalamus and pituitary: implications for control of hormone synthesis and release. *Peptides* **5,** Suppl. 1, 121–138.

Herkenham M., Rice R. C., Jacobsen R. E., and Rothman R. B. (1986) Opiate receptors in the rat pituitary are confined to the neural lobe and are exclusively kappa. *Brain Res.* **382,** 365–371.

Hökfelt T., Wiesenfeld-Hallin Z., Villar M. J., and Melander T. (1987) Increase of galanin-like immunoreactivity in rat dorsal root ganglion cells after peripheral axotomy. *Neurosci. Lett.* **83,** 217–220.

Hou-Yu A., Lamme A. T., Zimmerman E. A., and Silverman A. J. (1986) Comparative distribution of vasopressin and oxytocin neurons in the rat brain and spinal cord. *Neuroendocrinology* **44,** 235–246.

Hulting A.-L., Meister B., Carlsson L., Hilding A., and Isaksson O. (1991) On the role of the peptide galanin in regulation of growth hormone secretion. *Acta Endocrinol.* **125,** 518–525.

Kalimo H. (1975) Ultrastructural studies on the hypothalamic neurons of the rat. III. Paraventricular and supraoptic neurons during lactation and dehydration. *Cell Tissue Res.* **163,** 151–168.

Kaplan L. M., Gabriel S. M., Koenig J. I., Sunday M. E., Spindel E. R., Martin J. B., and Chin W. W. (1988) Galanin is an estrogen-inducible, secretory product of the rat anterior pituitary. *Proc. Natl. Acad. Sci. USA* **85,** 7408–7412.

Kato Y., Iwasaki Y., Iwasaki J., Abe H., Yanaihara H., Nakajima T., and Ibata Y. (1978) Prolactin release by vasoactive intestinal polypeptide in rats. *Endocrinology* **103,** 554–558.

Kiss J. Z, Mezey E., and Skirboll L. (1984) Corticotropin-releasing factor-immunoreactive neurons of the paraventricular nucleus become vasopressin positive after adrenalectomy. *Proc. Natl. Acad. Sci. USA* **81,** 1854–1858.

Kiss J. Z. and Mezey E. (1986) Tyrosine hydroxylase in magnocellular neurons: response to physiological manipulations. *Neuroendocrinology* **43,** 519–525.

Kitajima N., Chihara K., Abe H., Okimura Y., and Shakutsui S. (1990) Galanin stimulates immunoreactive growth hormone-releasing factor secretion from rat hypothalamic slices perifused in vitro. *Life Sci.* **47,** 2371–2376.

Kiyama H., Emson P. C. (1990) Evidence of the coexpression of oxytocin and vasopressin messenger ribonucleic acids in magnocellular neurosecretory cells: simultaneous demonstration of two neurophysin messenger ribonucleic acids by hybridization histochemistry. *J. Neuroendocrinol.* **2,** 257–259.

Koenig J. I., Hooi S., Gabriel S. M., and Martin J. B. (1989) Potential involvement of galanin in the regulation of fluid homeostasis in the rat. *Regul. Peptides.* **24,** 81–86.

Locatelli V., Torsello A., Redaelli M., Ghigo E., Massara F., and Müller E. E. (1986) Cholinergic agonist and antagonist drugs mediate the GH response to GHRH in the rat: evidence for mediation by SRIF. *J. Endocrinol.* **111,** 271–278.

Lorens R. G., Evans C. J., and Barchas J. D. (1985) Effects of dehydration on pro-dynorphin derived peptides in the neuro-intermediate lobe of the rat pituitary. *Life Sci.* **37,** 1523–1528.

MacLeod R. M. and Lehmayer J. E. (1974) Studies on the mechanism of dopamine-mediated inhibition of prolactin secretion. *Endocrinology* **94,** 1077–1085.

Maeda K. and Frohman L. A. (1978) Dissociation of systemic and central effects of neurotensin on the secretion of growth hormone, prolactin and thyrotropin. *Endocrinology* **103,** 1903–1909.

Martin R. and Voigt K. H. (1981) Enkephalin coexists with oxytocin and vasopressin in nerve terminals of rat neurohypophysis. *Nature (Lond.)* **289,** 502–504.

Martin R., Geis R., Holl R. Schäfer M., and Voigt K. H. (1983) Coexistence of unrelated peptides in oxytocin and vasopressin terminals of rat neurohypophysis: immunoreactive methionine^5-enkephalin, leucine^5-enkephalin, and cholecystokinin-like substances. *Neuroscience* **8,** 213–227.

McCann S. M. (1982) The role of brain peptides in the control of anterior pituitary hormone secretion, in *Neuroendocrine Perspectives* (Müller E. E. and MacLeod R. M., eds.), Elsevier, Amsterdam, pp. 1–22.

McCann S. M. and Rettori V. (1986) Gamma-aminobutyric acid (GABA) controls anterior pituitary hormone secretion, in *GABA and Endocrine Function* (Racagni G. and Donoso A. O., eds.), Raven, New York, pp. 172–189.

Meeker R. B., Swanson D. J., and Hayward J. N. (1989) Light and electron microscopic localization of glutamate immunoreactivity in the supraoptic nucleus of the rat hypothalamus. *Neuroscience* **33,** 157–167.

Meister B. (1991) Monosodium glutamate (MSG) lesions, in *Methods in Neurosciences, vol. 7, Transplantation, Lesions, and Surgical Techniques* (Conn P. M., ed.), Academic Press, Orlando, FL, Chapter 6, pp. 70–89.

Meister B. and Hulting A.-L. (1987) Influence of coexisting hypothalamic messengers on growth hormone secretion from rat anterior pituitary cells in vitro. *Neuroendocrinology* **46,** 387–394.

Meister B. and Hökfelt T. (1988) Peptide- and transmitter-containing neurons in the mediobasal hypothalamus and their relation to GABAergic systems: possible roles in control of prolactin and growth hormone secretion. *Synapse* **2,** 585–605.

Meister B. and Hökfelt T. (1992) The somatostatin and growth hormone-releasing factor-containing systems, in *Neuroendocrinology* (Nemeroff C. B., ed.), CRC, Boca Raton, FL, Chapter 9, pp. 219–278.

Meister B., Hökfelt T., Steinbusch H. W. M., Skagerberg G., Lindvall O., Geffard M., Joh T. H., Cuello A. C., and Goldstein M. (1988a) Do tyrosine hydroxylase-immunoreactive neurons in the ventrolateral arcuate nucleus produce dopamine or only L-DOPA? *J. Chem. Neuroanatomy* **1,** 59–64.

Meister B., Hökfelt T., Geffard M., and Oertel W. (1988b) Glutamic acid decarboxylase and gamma-aminobutyric acid-immunoreactivities in corticotropin-releasing factor containing parvocellular neurons of the hypothalamic paraventricular nucleus. *Neuroendocrinology* **48,** 516–526.

Meister B., Ceccatelli S., Hökfelt T., Andén N.-E., Andén M., and Theodorsson E. (1989) Neuropeptides, neuropeptides and binding sites in the rat mediobasal hypothalamus: effects of monosodium glutamate (MSG) lesions. *Exp. Brain Res.* **76,** 343–368.

Meister B., Scanlon M. F., and Hökfelt T. (1990a) Occurence of galanin-like immunoreactivity in growth hormone-releasing factor (GRF)-containing neurons in the monkey *(Macaca fascicularis)* infundibular nucleus and median eminence. *Neurosci. Lett.* **119,** 136–139.

Meister B., Cortés R., Villar M. J., Schalling M., and Hökfelt T. (1990b) *Peptides* and transmitter enzymes in hypothalamic magnocellular neurons after administration of hyperosmotic stimuli: comparison between messenger RNA and peptide/protein levels. *Cell Tissue Res.* **260,** 279–297.

Meister B., Villar M. J., Ceccatelli S., and Hökfelt T. (1990c) Localization of chemical messengers in magnocellular neurons of the hypothalamic supraoptic and paraventricular nuclei: an immunohistochemical study using experimental manipulations. *Neuroscience* **37,** 603–633.

Meister B., Hulting A.-L., Uvnäs-Moberg K., and Hökfelt T. (1993) Galanin stimulates the release of cholecystokinin from the rat neural lobe. (submitted).

Meister B., Nicholas A. P., and Hökfelt T. (1993) Glutamate/asapartate-like immunoreactivity in chemically identified neurons of the Hypothalamus (in manuscript).

Melander T., Fuxe K., Härfstrand A., Eneroth P., and Hökfelt T. (1987) Effects of intraventricular injections of galanin on neuroendocrine functions in the male rat: possible involvement of hypothalamic catecholamine nerve terminal systems. *Acta Physiol. Scand.* **131,** 25–32.

Merchenthaler I., Lopez F. J., and Negro-Vilar, A. (1990) Colocalization of galanin and luteinizing hormone-releasing hormone (LHRH) in a subset of preoptic neurons. *Proc. Natl. Acad. Sci. USA* **87,** 6326–6330.

Merchenthaler I., Lopez F. J., Lennard, D. E., and Negro-Vilar, A. (1991) Sexual differences in the distribution of neurons coexpressing galanin and luteinizing hormone-releasing hormone in the rat brain. *Endocrinology* **129,** 1977–1986.

Mezey E. and Kiss J. Z. (1991) Coexpression of vasopressin and oxytocin in hypothalamic supraoptic neurons of lactating rats. *Endocrinology* **129,** 1814–1820.

Mohr E., Bahnsen V., Kiessling C., and Richter D. (1988) Expression of the vasopressin and oxytocin genes in rats occurs in mutually exclusive sets of hypothalamic neurons. *FEBS Lett.* **242,** 144–148.

Murakami Y., Kato Y., Koshiyama H., Inoue T., Yanaihara N., and Imura H. (1987) Galanin stimulates growth hormone (GH) secretion via GH-releasing factor (GRF) in conscious rats. *Eur. J. Pharmacol.* **136,** 415–418.

Murakami Y., Kato Y., Shimatsu A., Koshiyama H., Hattori N., Yanaihara N., and Imura H. (1989) Possible mechanism involved in growth hormone secretion induced by galanin in the rat. *Endocrinology* **124,** 1224–1229.

Okamura H., Kitahama K., Nagatsu I., and Geffard M. (1988a) Comparative topography of dopamine- and tyrosine hydroxylase-immunoreactive neurons in the rat arcuate nucleus. *Neurosci. Lett.* **95,** 347–353.

Okamura H., Kitahama K., Raynaud B., Nagatsu I., Borri-Voltatorni C., and Weber M. (1988b) Aromatic L-amino acid decarboxylase (AADC)-immunoreactive cells in the tuberal region of the rat hypothalamus. *Biomed. Res.* **9,** 261–267.

Olney J. W. (1969) Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. *Science* **164,** 719–721.

Ottlecz A., Samson W. K., and McCann S. M. (1986) Galanin: evidence for a hypothalamic site of action to release growth hormone. *Peptides* **7,** 51–53.

Ottlecz A., Snyder G. D., and McCann S. M. (1988) Regulatory role of galanin in control of hypothalamic pituitary function. *Proc. Natl. Acad. Sci. USA* **85,** 9861–9865.

Palkovits M., Lang T., Patthy A., and Elekes I. (1986) Distribution and stress-induced increase of glutamate and aspartate levels in discrete brain nuclei of rats. *Brain Res.* **373,** 252–257.

Peña P., Rodriguez E. M., Dellmann H. D., and Schoebitz K. (1988) Effects of colchicine on the hypothalamo-neurohypophysial system of chronically salt-loaded rats. *Neuroendocrinology* **47,** 217–224.

Quabbe H. J. (1986) Growth hormone, in *Neuroendocrinology* (Lightman S. L. and Everitt B. J., eds.), Blackwell, London, pp. 409–449.

Racagni G., Apud J. A., Cocchi D., Locatelli V., and Muller E. E. (1982) GABAergic control of anterior pituitary hormone secretion. *Life Sci.* **31,** 823–838.

Rhodes C. H., Morell J. I., and Pfaff D. W. (1981) Immunohistochemical analysis of magnocellular elements in rat hypothalamus: distribution and number of cells containing neurophysin, oxytocin and vasopressin. *J. Comp. Neurol.* **223,** 556–582.

Rivier C., Brown M., and Vale W. (1977) Effect of neurotensin, substance P and morphine sulfate on the secretion of prolactin and growth hormone in the rat. *Endocrinology* **100,** 751–754.

Rökaeus Å., Young W. S. III, and Mezey E. (1988) Galanin coexists with vasopressin in the normal rat hypothalamus and galanin's synthesis is increased in the Brattleboro (diabetes insipidus) rat. *Neurosci. Lett.* **90,** 45–50.

Ruberg M., Rotsztejn W. H., Aranciba S., Besson J., and Enjalbert A. (1978) Stimulation of prolactin release by vasoactive intestinal polypeptide. *Eur. J. Pharmacol.* **51,** 319–320.

Sawchenko P. E., Swanson L. W., and Vale W. W. (1984) Coexpression of corticotropin-releasing factor- and vasopressin immunoreactivity in parvocellular neurosecretory neurons of the adrenalectomized rat. *Proc. Natl. Acad. Sci. USA* **81,** 1883–1887.

Sawchenko P. E., Swanson L. W., Rivier J., and Vale W. W. (1985) The distribution of growth hormone-releasing factor (GRF)-immunoreactivity in the central nervous system of the rat: an immunohistochemical study using antisera directed against rat hypothalamic GRF. *J. Comp. Neurol.* **237,** 100–115.

Scharrer E., and Scharrer B. (1954) Hormones produced by neurosecretory cells. *Rec. Progr. Horm. Res.* **10,** 183–240.

Sherman T. G., McKelvy J. F., and Watson S. J. (1986a) Vasopressin mRNA regulation in individual hypothalamic nuclei: a northern and *in situ* hybridization study. *J. Neurosci.* **6,** 1684–1694.

Sherman T. G., Civelli O., Douglass J., Herbert E., and Watson S. J. (1986b) Coordinate expression of hypothalamic pro dynorphin and pro vasopressin with osmotic stimulation. *Neuroendocrinology* **44,** 222–228.

Sherman T. G., Day R., Civelli O., Douglass J., Herbert E., Akil H., and Watson S. J. (1988) Regulation of hypothalamic magnocellular neuropeptides and their mRNAs in the Brattleboro rat: coordinate responses to further osmotic challenge. *J. Neurosci.* **8,** 3785–3796.

Skofitsch G., Jacobowitz D. M., Amann R., and Lembeck F. (1989) Galanin and vasopressin coexist in the rat hypothalamo-neurohypophyseal system. *Neuroendocrinology* **49,** 419–427.

Swanson L. W., Sawchenko P. E., and Lind R. W. (1986) Regulation of multiple peptides in CRF parvocellular neurosecretory neurons: implications for the stress response. *Progr. Brain Res.* **68,** 169–190.

Tramu G., Croix C., and Pillez A. (1983) Ability of parvocellular CRF immunoreactive neurons of the paraventricular nucleus to produce a vasopressin-like material. *Neuroendocrinology* **37,** 467–469.

Tsuruo Y., Ceccatelli S., Villar M. J., Hökfelt T., Visser T. J., Terenius L., Goldstein M., Brown J. C., Buchan A., Walsh J., Morris M., Sofroniew M. V., and Verhofstad A. (1988) Coexistence of TRH with other neuroactive substances in the rat central nervous system. *J. Chem. Neuroanat.* **1,** 235–253.

Van den Pol A. N., Wuarin J-P., and Dudek F. E. (1990) Glutamate, the dominant excitatory transmitter in neuroendocrine regulation. *Science* **250,** 1276–1278.

Van den Pol, A. N. (1991) Glutamate and aspartate immunoreactivity in hypothalamic presynaptic neurons. *J. Neurosci.* **11,** 2087–2101

Van Tol H. H. M., Voorhuis D. Th. A. M., and Burbach J. P. H. (1987) Oxytocin gene expression in discrete hypothalamic magnocellular cell groups is stimulated by prolonged salt loading. *Endocrinology* **120,** 71–76.

Vanderhaeghen J. J., Lotstra F., Vandersande F., and Dierickx K. (1981) Coexistence of cholecystokinin and oxytocin-neurophysin in some magnocellular hypothalamo-hypophyseal neurons. *Cell Tissue Res.* **221,** 227–231.

Vanderhaeghen J. J., Lotstra F., Liston D. R., and Rossier J. (1983) Proenkephalin, (Met)enkephalin and oxytocin immunoreactivities are colocalized in bovine hypothalamic magnocellular neurons. *Proc. Natl. Acad. Sci. USA* **80,** 5139–5143.

Vijayan E. and McCann S. M. (1979) Effects of substance P and neurotensin on gonadotropin and prolactin release. *Endocrinology* **105,** 64–68.

Villar M. J., Cortés R., Theodorsson E., Wiesenfeld-Hallin Z., Schalling M., Fahrenkrug J., Emson P., and Hökfelt T. (1989) Neuropeptide expression in the dorsal root ganglia and dorsal horn of the spinal cord after peripheral nerve injury with special reference to galanin. *Neuroscience* **33,** 587–604.

Villar M. J., Meister B., Cortés R., Schalling M., Morris M., and Hökfelt T. (1990) Neuropeptide gene expression in hypothalamic magnocellular neurons of normal and hypophysectomized rats: a combined immunohistochemical and *in situ* hybridization study. *Neuroscience* **36,** 181–199.

Vrontakis M. E., Yamamoto T., Schroedter I. C., Nagy J. I., and Friesen H. G. (1989) Estrogen induction of galanin synthesis in the rat anterior pituitary gland demonstrated by *in situ* hybridization and immunohistochemistry. *Neurosci. Lett.* **100,** 59–64.

Watson S. J., Akil H., Fischli W., Goldstein A., Zimmerman E., Nilaver G., and Van Wimersma Greidanus T. B. (1982) Dynorphin and vasopressin: common localization in magnocellular neurons. *Science* **216,** 85–87.

Watts A. G. (1992) Disturbance of fluid homeostasis leads to temporally and anatomically distinct responses in neuropeptide and tyrosine hydroxylase mRNA levels in the paraventricular and supraoptic nuclei of the rat. *Neuroscience* **46,** 859–879.

Whitnall M. H., Gainer H., Cox B. M., and Molineaux C. J. (1983) Dynorphin-A (1–8) is contained within vasopressin neurosecretory vesicles in the rat. *Science* **222,** 1137–1139.

Young W. S. III (1986) Corticotropin-releasing factor mRNA in the hypothalamus is affected differently by drinking saline and by dehydration. *FEBS Lett.* **208,** 158–162.

Young W. S. III, Mezey E., and Siegel R. E. (1986) Quantitative *in situ* hybridization histochemistry reveals increased levels of corticotropin-releasing factor mRNA after adrenalectomy in rats. *Neurosci. Lett.* **70,** 198–203.

Young W. S. III, Warden M., and Mezey E. (1987) Tyrosine hydroxylase mRNA is increased by hyperosmotic stimuli in the paraventricular and supraoptic nuclei. *Neuroendocrinology* **46,** 439–444.

Young W. S. III, Horváth S., and Palkovits M. (1990) The influences of hyperosmolarity and synaptic inputs on galanin and vasopressin expression in the hypothalamus. *Neuroscience* **39,** 115–125.

Zerbe R. L. and Palkovits M. (1984) Changes in the vasopressin content of discrete brain regions in response to stimuli for vasopressin secretion. *Neuroendocrinology* **38,** 285–289.