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THE NEUROENDOCRINE PARAVENTRICULAR HYPOTHALAMUS: RECEPTORS, SIGNAL TRANSDUCTION, mRNA AND NEUROSECRETION

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Summary

The hypothalamus is one of the most studied areas of the central nervous system. Many of its functions are understood and there is an extensive literature on its role in the control of pituitary hormone secretion, autonomic nervous system activity, regulation of salt, water and food ingestion, body temperature regulation and aspects of behaviour. Although the role of the hypothalamus in the control of pituitary secretion was postulated in the early 1900s, the chemical nature of these control mechanisms has only been documented in the last few years.

The opioid peptides represent one particular family of chemical compounds which have been shown to have many effects on pituitary hormone secretion. Exogenous opioids inhibit the neurosecretion of both vasopressin and oxytocin from the posterior pituitary neurosecretory terminals of hypothalamic cell bodies. Opioids also have major actions on the secretory activity of the anterior pituitary which has no innervation from the hypothalamus, but which is regulated by blood-borne factors in the hypophyseal portal circulation which runs from the median eminence of the hypothalamus. It was therefore of considerable interest when it was discovered that endogenous opioid peptides could be detected both in the neurohypophyseal system and in cells which project into the median eminence.

The simple presence of a peptide in a neurone does not necessarily imply a function. If, however, we can demonstrate that regulation of the synthesis of the peptide occurs in a manner which corresponds with the expected role of the agent, this provides powerful data in support of a genuine physiological function. The elucidation of the genomic structure of the precursors for the three endogenous opioid peptides has provided us with the ability to measure mRNA for these peptides in defined areas of the brain and to assess their response to appropriate stimuli. Not only does mRNA for the endogenous opioid dynorphin coexist in the same cells as vasopressin but we have now been able to demonstrate that stimuli to vasopressin secretion also result in a markedly increased accumulation of dynorphin mRNA. Similarly, previous studies have shown that opioid peptides derived from another precursor – pro-enkephalin A – coexist with corticotrophin releasing factor in a different group of hypothalamic cells. We have now been able to demonstrate that stresses which result in an accumulation of corticotrophin

Key words: opioids, stress, *in situ* hybridization, hypothalamus.

releasing factor mRNA also result in increased pro-enkephalin mRNA in the same area. This considerably strengthens the hypothesis that endogenous opioids do play a significant role in the control of hypophyseal secretion.

The ability to perform quantitative assessments of mRNA in anatomically localized areas of the central nervous system can be applied to mRNA for other products of gene transcription including the mRNA for receptors. Acetylcholine is a classic neurotransmitter which has long been known to stimulate the release of vasopressin. A probe complementary to part of the rat nicotinic receptor sequence was found to hybridize to the vasopressin-containing areas of the hypothalamus which project to the neurohypophysis, and indeed the expression of this mRNA could be increased by a stimulus to vasopressin secretion.

Quantitative studies of mRNA should provide a powerful tool for investigating the control of transmitter and receptor physiology at any site in the body which responds to its local chemical environment.

Introduction

The hypothalamic paraventricular (PVN) and supraoptic (SON) nucleus complex is an excellent model system for the study of the control of neuroendocrine neurones, from receptor activation to mRNA transcription. This system consists of two major divisions (Fig. 1) whose functions have been clearly described.

First, there is the parvocellular system of the PVN which, together with fibres from other areas of the hypothalamus, projects to the external zone of the median eminence of the hypothalamus. This area abuts onto a rich capillary network which carries blood in the hypothalamo-pituitary portal system to the anterior pituitary gland where agents in the portal blood can modify the secretory activity of anterior pituitary cells. Portal blood contains many biologically active compounds but the most important factors in anterior pituitary regulation include not only corticotrophin releasing factor (CRF) and arginine vasopressin (AVP) from the parvocellular PVN which stimulate the release of adrenocorticotrophin (ACTH), but also thyrotrophin releasing hormone (TRH) which releases thyrotrophin (TSH) and prolactin, gonadotrophin releasing hormone (GnRH) which releases luteinizing hormone (LH) and follicle stimulating hormone (FSH), growth hormone releasing factor (GHRH) which releases growth hormone (GH), somatostatin which inhibits the release of GH and to a lesser extent TSH, and dopamine which inhibits the release of prolactin. Portal blood also contains vasoactive intestinal peptide, neuropeptide Y, enkephalin, neurotensin, adrenaline, noradrenaline, γ-aminobutyric acid (GABA) and several other compounds whose physiological role remains uncertain. Since the cells of origin of all these pituitary hormone releasing and inhibitory factors have been clearly defined we are in a good situation to study the mechanisms underlying their control.

The second system shown in Fig. 1 is the magnocellular system based both in the SON and PVN, whose axons run to the neural lobe of the pituitary whence neurosecretion occurs directly into the venous circulation. These pathways primarily secrete AVP, the antidiuretic hormone which acts on the kidney to

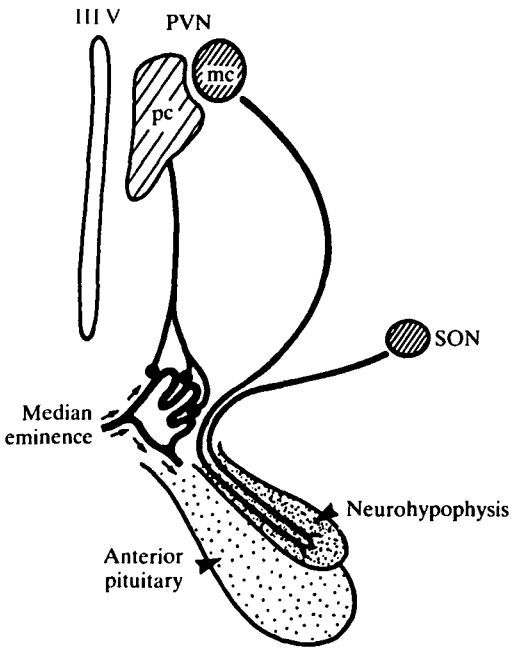


Fig. 1. Schematic representation of the rat hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei. The SON and magnocellular (mc) division of the PVN project to the neurohypophysis, and the parvocellular (pc) division of the PVN projects to the capillary bed of the median eminence. III V is the third ventricle.

conserve water and also has pressor effects on blood vessels, and oxytocin (OT), a hormone which causes contraction of the smooth muscle responsible for the milk let-down during suckling and the uterine muscle responsible for foetal expulsion during parturition.

There is a third system in many animals (but not in man) consisting of a separate intermediate lobe of secretory cells which make pro-opiomelanocortin-derived peptides whose activity is under direct neural regulation. I shall not discuss this system any further in this chapter.

The parvocellular system

The parvocellular hypothalamus is extremely complex and has many neuroendocrine, autonomic and behavioural roles. I shall limit myself to the neuroendocrine response to stress and the possible contribution of endogenous opioid peptides.

Studies with stress and exogenous opioids

The median eminence is rich in opioid receptors (Atweh & Kuhar, 1977) and clearly represents a site at which endogenous opioid peptides could have far-

ranging actions on a large number of pituitary releasing or inhibitory factors. Stressful stimuli and the administration of exogenous opioids have many, and often very similar, effects on pituitary hormone secretion. ACTH, a classic pituitary hormone released during stress, is also released by intrahypothalamic microinjections of morphine (Lotti, Kokka & George, 1969). Methionine and leucine enkephalin have both been demonstrated to cause a dose-dependent increase in corticotrophin releasing factor release from rat hypothalamus *in vitro* (Buckingham & Cooper, 1984), suggesting a functional role for hypothalamic enkephalin release in the control of ACTH. Methionine enkephalin has also been demonstrated to increase plasma prolactin concentration, an effect that can be blocked by naloxone (Bruni, Van Vugt, Marshall & Meites, 1977), and naloxone itself can also suppress the prolactin release which occurs in response to stress (Grandison & Guidotti, 1977; Rossier *et al.* 1980). This action is likely to be secondary to the known effect of opioids in inhibiting the release of the prolactin inhibiting factor dopamine from tuberoinfundibular neurones (Gudelsky & Porter, 1979).

Stress has an inhibitory effect on three other anterior pituitary hormones: LH, TSH and GH. Methionine enkephalin has been demonstrated to inhibit the dopamine-induced release of the LH releasing factor LHRH *in vitro* (Rotsztejn, Drouva, Pattou & Kordon, 1978), and the opioid antagonist naltrexone reverses the inhibition of LH secretion induced by stress (Briski, Quigley & Meites, 1984*a*,*b*). Hypothalamic lesion studies also reveal that opioid-induced suppression of TSH secretion involves an action at the level of the hypothalamus (Lomax & George, 1966), and there is evidence that endogenous opioids are involved in stress-mediated inhibition of TSH release (Muraki, Nakadate, Tokunada & Kato, 1980). Unlike ACTH, prolactin, LH and TSH, the stress-induced fall of GH appears to result from a non-opioid-dependent mechanism (Briski, Quigley & Meites, 1984*a*,*b*), and indeed not only do opioid peptides result in a stimulation of GH secretion (Bruni *et al.* 1977) but they have now been shown to diminish the release of somatostatin from rat hypothalamus *in vitro* (Drouva *et al.* 1981) and to stimulate the activity of GHRH (Miki, Ono & Shizume, 1984).

Parvocellular enkephalin neurones may also modulate the posterior pituitary response to stress. Although the cell bodies of the vasopressin and oxytocin cells are in the adjacent magnocellular division of the paraventricular nucleus (see next section of chapter) they have a rich dendritic tree within the parvocellular division of this nucleus (Van den Pol, 1982). It is notable that the opiate antagonist naloxone has no effect on basal vasopressin or oxytocin secretion, but it does potentiate the vasopressin and oxytocin response to stress (Carter, Williams & Lightman, 1986).

It is clear, therefore, that there is a close relationship between the neuroendocrine effects of exogenous opioids and stress-mediated responses. The question which must therefore be answered is whether stress itself alters the regulation of endogenous opioid peptides.

Studies of endogenous opioid peptides

Immunocytochemical studies have demonstrated the presence of enkephalin immunoreactive neurones in the medial parvocellular subdivision of the paraventricular nucleus (Hokfelt *et al.* 1983; Khachaturian, Lewis & Watson, 1983; Merchenthaler, Maderdrut, Altschuler & Petrusz, 1986; Fallon & Leslie, 1986). In addition, there is evidence for coexistence of enkephalin with CRF both in parvocellular paraventricular cells (Hokfelt *et al.* 1983) and in granules in the median eminence (Hisano, Daikoku, Yanaihara & Shibasaki, 1986). Since CRF is an important releasing factor for the stress hormone ACTH, the coexistence of CRF with enkephalin further supported the hypothesis that endogenous enkephalin may have a role in the neuroendocrine response to stress.

CRF is found in both the magnocellular and parvocellular divisions of the PVN, and there have been conflicting data as to whether enkephalin existed in the magnocellular as well as the parvocellular cells of this nucleus (see under discussion of magnocellular system). It is important, therefore, when designing a study to look at the control of CRF and enkephalin mRNA to use a technique which can differentiate between the magnocellular and parvocellular subdivisions of the small paraventricular nucleus in the hypothalamus. A tissue-punching technique would not be accurate enough, but quantitative *in situ* hybridization histochemistry does provide the necessary anatomical specificity. A schematic representation of the technique is given in Fig. 2. Basically the brain is removed rapidly, placed in dry ice and sections are cut through the appropriate area. After fixing and delipidating, the sections are incubated with hybridization buffer containing a labelled probe complementary to the mRNA of interest, washed and then put on X-ray film or under photographic emulsion. The developed film can then be visualized, photographed and submitted to quantitative image analysis. The detailed aspects of this technique are described by Young, Mezey & Siegel (1986).

We have used this technique to study the response of the hypothalamus to two stressful stimuli: naloxone-precipitated opiate withdrawal in the morphine-dependent rat; and intraperitoneal hypertonic saline injection (Lightman & Young, 1987*a*). The response of parvocellular paraventricular enkephalin and CRF mRNA can be seen in Fig. 3. There is clearly a marked increase in CRF mRNA in response to both stresses. In the control animals we could only detect a few scattered cells containing enkephalin mRNA. In the naloxone-precipitated withdrawal and intraperitoneal hypertonic saline-injected animals, however, there was a rapid and massive accumulation of enkephalin mRNA.

The combination of a rapid activation of enkephalin mRNA in response to stress, major effects of exogenous opioids on pituitary hormone secretion and similar hormone responses to stress all suggest that endogenous opioids may play an important role in these responses. Clearly, further studies will be needed to discriminate which effects are directly attributable to activation of enkephalin transcription in these cells.

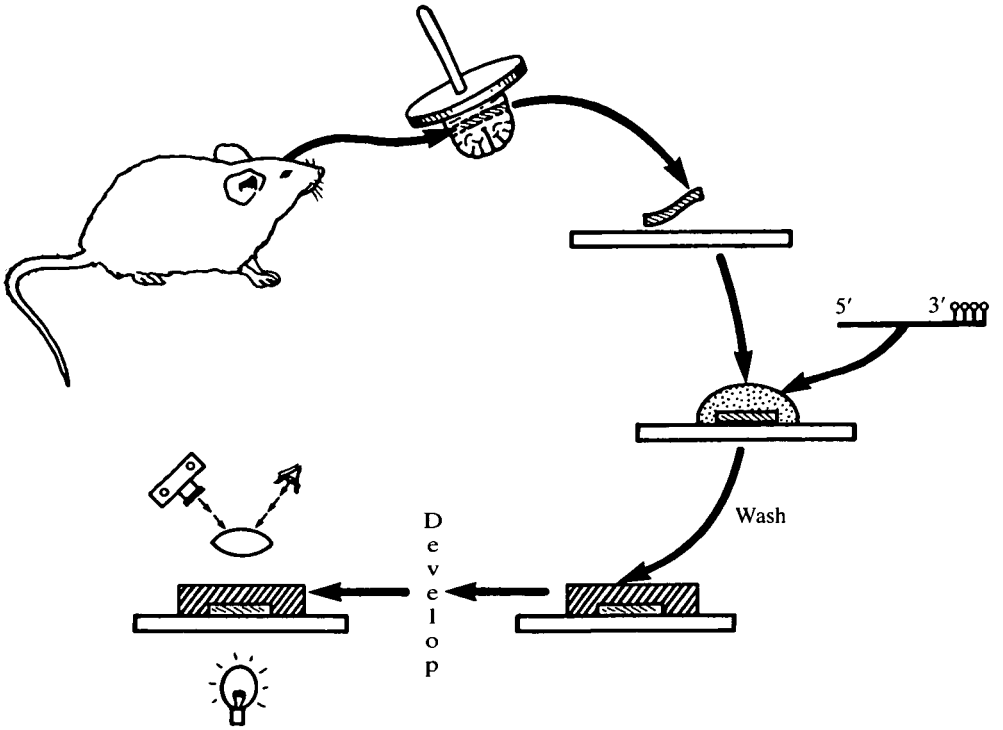


Fig. 2. Methodology of *in situ* hybridization histochemistry. The brain is removed, frozen on dry ice and mounted on a chuck. Sections are cut through the brain area of interest, thaw-mounted onto slides, fixed, delipidated, and incubated with incubation buffer containing a labelled probe complementary to the mRNA to be analysed. The sections are then washed at high stringency and apposed to film or dipped into photographic emulsion.

The magnocellular hypothalamo-neurohypophyseal system

The magnocellular neurosecretory system can be seen in more detail in Fig. 4. Basically there are two sets of neurones containing either vasopressin or oxytocin mingled within both the supraoptic and paraventricular nuclei. There is evidence that these cells also contain other neurally active peptides, notably opioid peptides. This situation is clearest for the vasopressin-containing cell bodies, where co-localization with dynorphin was first described by Watson *et al.* (1982), and in the neural lobe, where vasopressin has been found together with dynorphin 1-8 in vasopressin neurosecretory vesicles (Whitnall, Gainer, Cox & Molineaux, 1983). Although some early studies found enkephalin in rat magnocellular cells, this may have been due to cross-reactivity of the antisera with the dynorphin A-NH_2 terminus as more recent studies have failed to repeat these observations. At the neural lobe, however, oxytocin terminals and granules have been reported to contain proenkephalin-A-derived peptides (Martin *et al.* 1983) but the reason for this disparity remains unclear. A recent study suggests that some oxytocin cells also contain some dynorphin (Sawchenko & Levin, 1987) and perhaps the

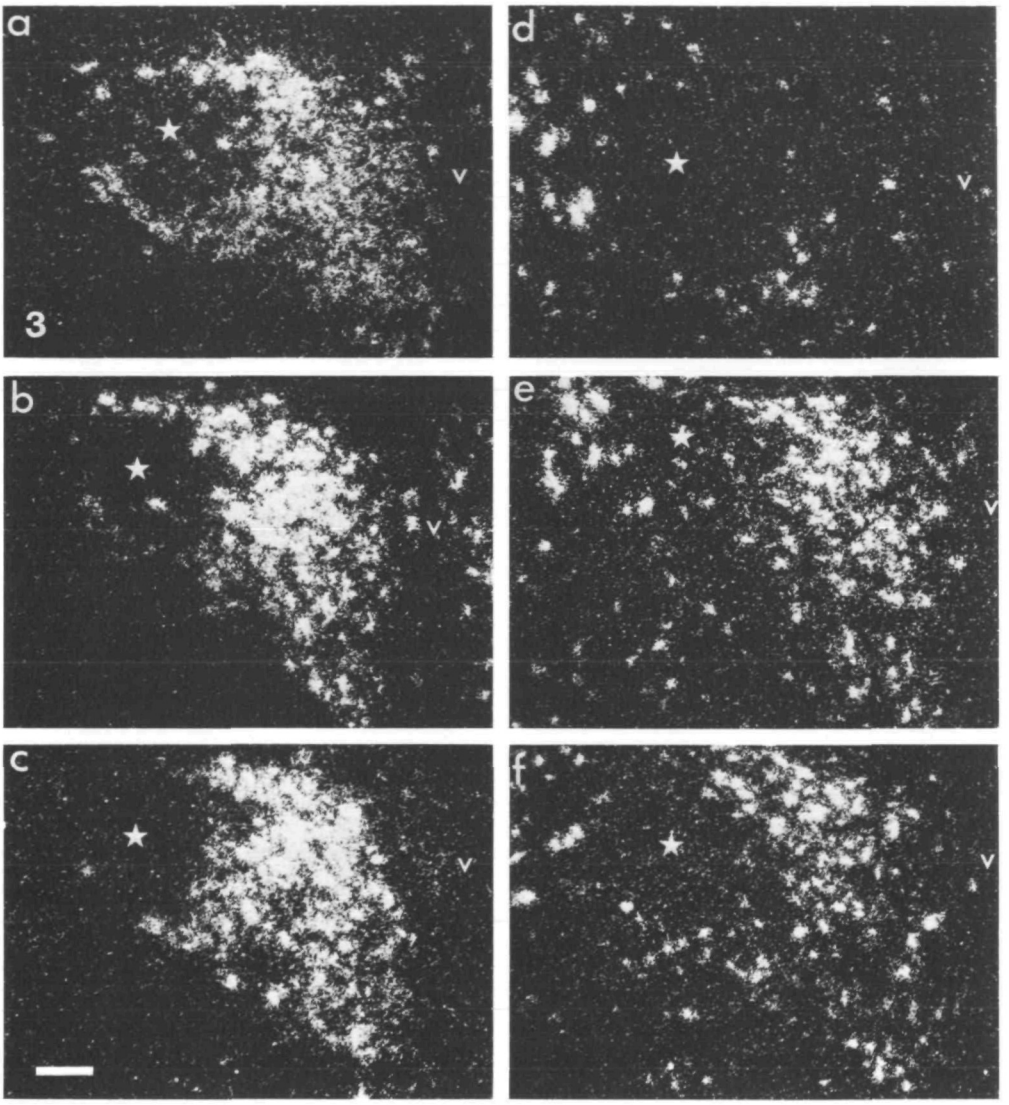


Fig. 3. Distribution of cells containing corticotrophin releasing factor (CRF) (a–c) and enkephalin (d–f) mRNA in the paraventricular nuclei from control (a,d), 24 h naloxone-precipitated withdrawal (b,e) and hypertonic-saline-treated (c,f) animals. The autoradiographic silver grains are white in these dark-field photomicrographs. Note the increase in CRF and enkephalin mRNA in parvocellular neurones between the magnocellular core (star) and the third ventricle (v). Labelled perifornical enkephalin cells (left of star) were seen in all conditions. Scale bar, 100 μm. (From Lightman & Young, 1987*a*.)

differential processing of this opioid peptide may result in products which cross-react with enkephalin antisera. Our own *in situ* hybridization histochemical studies (Lightman & Young, 1987*b*) confirm the presence of high levels of dynorphin

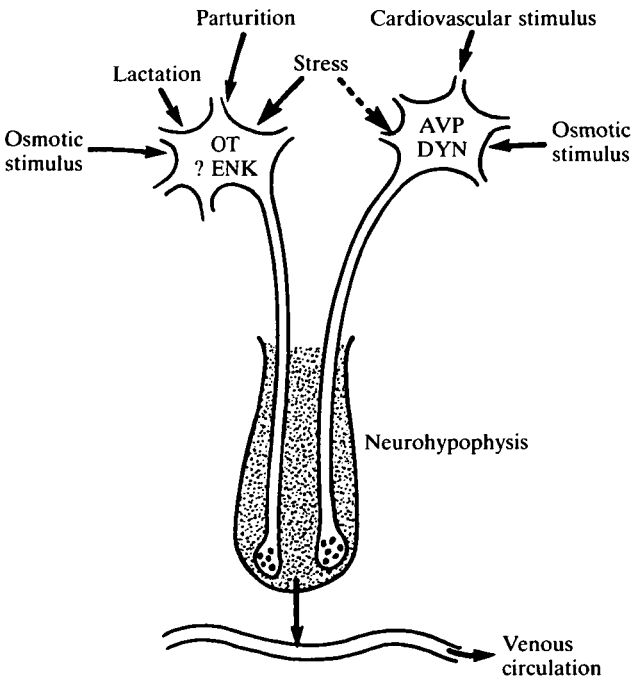


Fig. 4. The magnocellular system in the rat. Oxytocin (OT) and vasopressin (AVP) cells are adjacent to each other in the supraoptic and magnocellular paraventricular nuclei, and project to the neurohypophysis. Dynorphin (DYN) coexists with vasopressin, but whether oxytocin coexists with dynorphin or enkephalin (ENK) is unclear (see text).

mRNA in magnocellular cells, but only just detectable levels of proenkephalin transcripts, which would appear inconsistent with the high levels of proenkephalin peptide described in the neural lobe.

The neurosecretory terminals in the posterior pituitary are engulfed by specialized glial cells called pituicytes. Surprisingly, these cells have been shown to undergo rapid morphological changes associated with alterations in neural lobe neurosecretory activity (Hatton, Pearlmutter & Tweedle, 1984). Within a few hours of water deprivation the pituicytes withdraw their processes from the neurosecretory terminals, allowing increased contact of the terminals with the perivascular space and uncertain changes in the ionic environment. These glial cells may be under neural control – synaptoid contacts with opioid terminals have been described (Van Leeuwen & De Vries, 1983) – but no direct evidence of a functional relationship is yet available.

Pharmacological studies with exogenous opioids

In vitro studies have revealed opioid binding sites not only on the magnocellular PVN and SON (Atweh & Kuhar, 1983) but also in the neural lobe (Lightman, Ninkovic, Hunt & Iversen, 1983). As the predominant endogenous opioid

peptides in this system are likely to be dynorphin-related peptides which have relative selectivity for κ-opioid receptors, it is consistent with a physiological role for these agents that the neural lobe opioid binding sites are predominantly κ (Bunn, Hanley & Wilkin, 1985; Herkenham, Rice, Jacobson & Rothman, 1986; Gerstberger & Barden, 1986). The site of these receptors is uncertain. Both Lightman *et al.* (1983) and Bunn *et al.* (1985) demonstrated that the binding sites persist after destruction of the neurosecretory fibres and terminals and Pesce *et al.* (1987) have evidence that receptors may also be found on the neurosecretory terminals. The present evidence therefore suggests that neural lobe opiate receptors are predominantly κ and may exist on the neurosecretory terminals as well as on the pituicyte glial cells.

Electrophysiological studies have demonstrated an inhibitory effect of opioids on AVP-secreting cells in the SON (Clarke, Lincoln & Wood, 1980; Arnaud, Cirino, Layton & Renaud, 1983; Wakerley, Noble & Clarke, 1983) and on a proportion of paraventricular magnocellular neurones (Muehlethaler, Gaewiler & Dreifuss, 1980; Pittman, Hatton & Bloom, 1980). At the level of the neural lobe itself, opioids have been demonstrated to inhibit both oxytocin (Bicknell & Leng, 1982) and AVP release (Lightman, Iversen & Forsling, 1983) and Bicknell & Leng also demonstrated that the opioid antagonist naloxone could actually increase the electrically stimulated release of oxytocin. This suggests that endogenous opioids present within the neural lobe were actively inhibiting the release of oxytocin.

In vivo opiate antagonists have also been shown to increase the AVP response to electric shock (Knepel, Nutto & Hertting, 1982; Rosella-Dampman, Keil, Chee & Summy-Long, 1983), the oxytocin and vasopressin responses to immobilization stress in the rat (Fig. 5) (Carter *et al.* 1986) and the oxytocin response to vaginocervical stimulation in the goat (Seckl & Lightman, 1987). In man a naloxone infusion reveals an oxytocin response to insulin-induced hypoglycaemia which is not found after naloxone alone or hypoglycaemia alone (Dunne, Haddock, Lightman & Seckl, 1987). Clearly, endogenous opioids do seem to have a major inhibitory effect on oxytocin secretion across several species.

Studies with endogenous opioid peptides

In addition to the immunocytochemical evidence for co-localization of opioid peptides in magnocellular cells which project into the neural lobe, Millan, Millan & Herz (1983, 1984) have demonstrated that lesions to the PVN and SON result in a marked parallel fall of AVP and dynorphin levels in the neurointermediate lobe of the pituitary gland, a result that was also found following dehydration (Hollt, Haarmann, Seizinger & Herz, 1981). Dynorphin-like immunoreactivity can be released from neural lobes *in vitro* by potassium depolarization (Anhut & Knepel, 1982) but a relationship with vasopressin release was unclear. Studies have also been performed to see whether opioid antagonists have different effects during stimuli which elevate AVP (and presumably dynorphin release). Naltrexone has no effect on oxytocin secretion in normally hydrated animals but significantly elevates oxytocin levels in dehydrated or haemorrhaged animals (Summy-Long

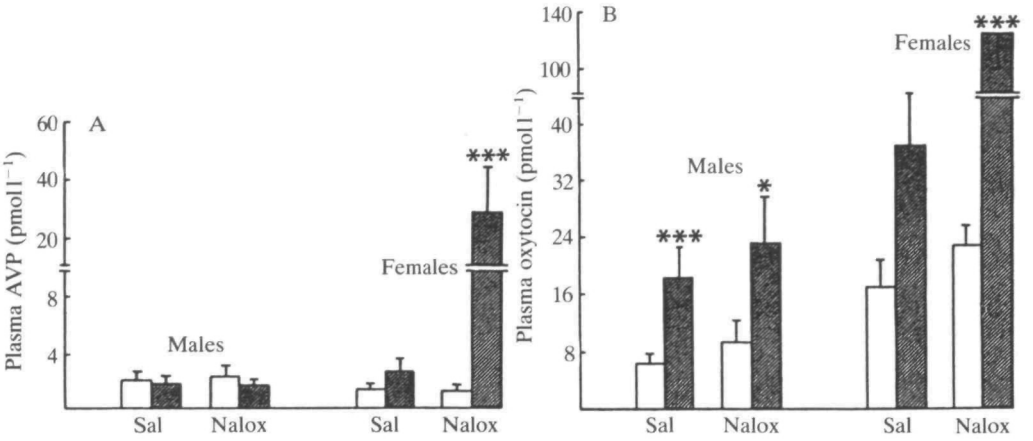


Fig. 5. Effect of an opiate antagonist on the neurohypophyseal response to stress. Plasma levels of (A) vasopressin (AVP) and (B) oxytocin in control (open bars) or 1-min immobilized (hatched bars) rats following acute treatment with either naloxone (Nalox) $1\,mg\,kg^{-1}$ subcutaneously or 0·9% saline vehicle (Sal). * $P<0{\cdot}05$; *** $P<0{\cdot}01$. (From Carter, Williams & Lightman, 1986.)

et al. 1984). These authors suggest that in these situations there is a preferential release of AVP due to the greater sensitivity of oxytocin terminals to co-released dynorphin. The differential control of vasopressin and oxytocin at the level of their terminals is certainly an interesting possible role for an endogenous opioid peptide. Prolonged stimulation of vasopressin secretion, however, not only reduces κ-opiate-receptor binding in the neurohypophysis (Brady & Herkenham, 1987) but also the naloxone-elicited rise in oxytocin level (Carter & Lightman, 1987), so that any effect of endogenously released dynorphin on oxytocin secretion only seems to occur at an early stage of dehydration.

I have cited the evidence correlating the concentration of posterior pituitary dynorphin with the presence of intact supraoptic and paraventricular nuclei in the hypothalamus, and pharmacological data linking the effects of opioid antagonists with the degree of activation of vasopressin terminals. We do not, however, have any evidence concerning the control of endogenous dynorphin biosynthesis. Osmotic stimulation increases the rate of synthesis of the AVP precursor (Gainer, Sarne & Brownstein, 1977). This increase in AVP synthesis necessitates an increase in translation of AVP mRNA and presumably an increase in AVP gene transcription. Several groups have studied this using probes of different sizes, ^{32}P, ^{3}H and ^{35}S labelling, cell-free translation, Northern analysis; densitometric and solution hybridization assays and *in situ* hybridization. These various techniques together with varied protocols have detected increases in AVP mRNA by factors of between 1·2 and 20. If dynorphin is indeed being co-released with AVP one would expect that a stimulus to AVP transcription would also act as a stimulus to dynorphin transcription. This has now been studied (Sherman, McKelvy & Watson, 1986; Lightman & Young, 1987*b*). Both groups found a marked similarity

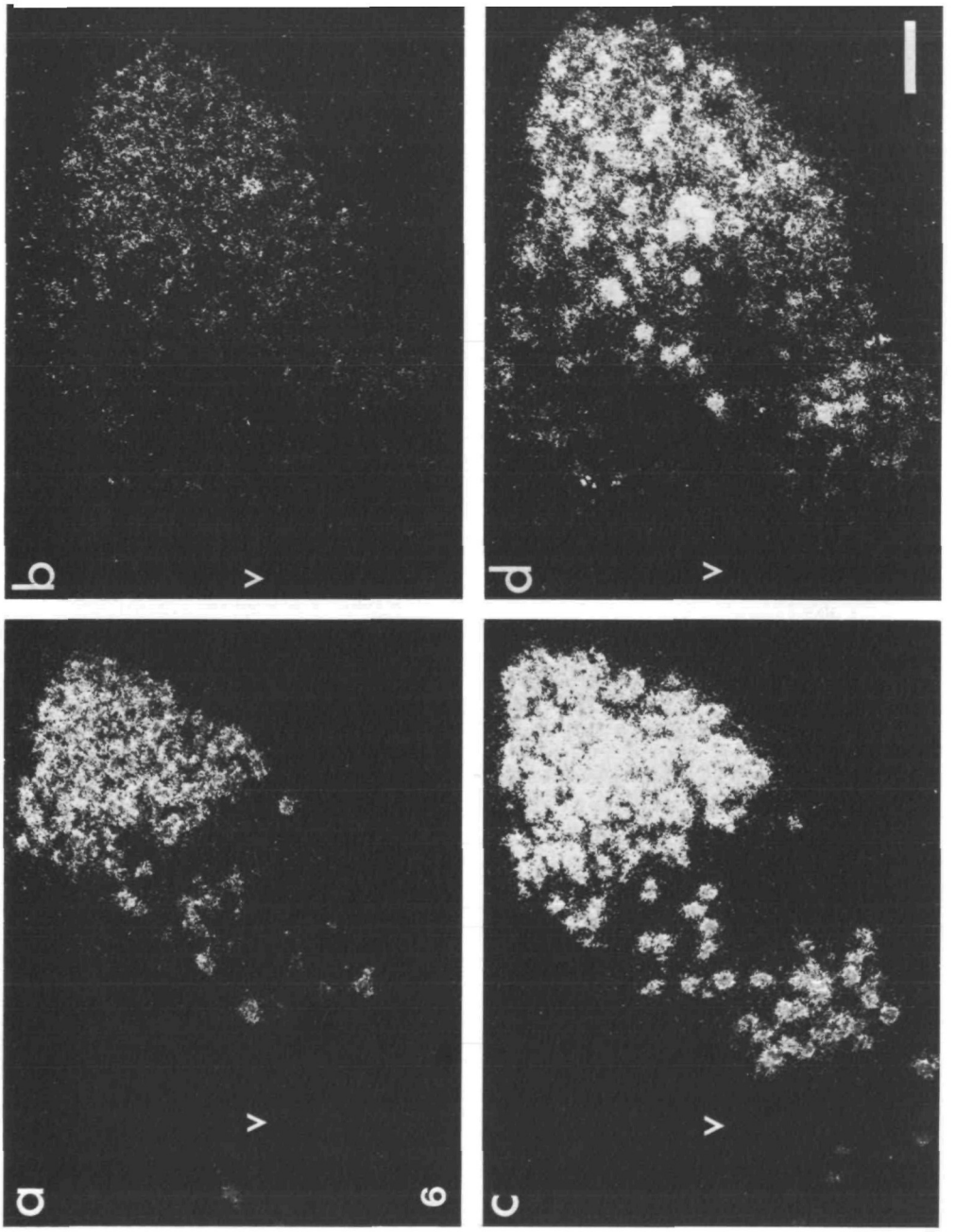


Fig. 6. Dark-field autoradiogram of *in situ* hybridization histochemical analysis of paraventricular magnocellular neurones. Cells contain vasopressin (a,c) and dynorphin (b,d) mRNAs in a control rat (a,b) and in a rat following the osmotic stimulus of salt loading (c,d). Note the increase in signal (silver grains) in the salt-loaded animals. v is the third ventricle. Scale bar, 100 μm. (From Lightman & Young, 1987*b*.)

in accumulation of AVP and dynorphin mRNAs in response to a hypertonic stimulus (see Fig. 6).

We have, therefore, now been able to link up our circle. Stimulation of vasopressin secretion results in an accumulation of both AVP and dynorphin mRNA and an increased synthesis of both peptides. We presume that the increased level of synthesis is linked to the increased release of dynorphin together with vasopressin. The major site of release of this dynorphin is presumably at the neural lobe where it preferentially inhibits oxytocin secretion, but probably also has some negative feedback effect on AVP release itself. Whether colaterals or other axons project back to the magnocellular cell bodies themselves or other opioid-sensitive sites in the central nervous system needs further study.

One further point that should be made is that the two populations of paraventricular nucleus opioids – dynorphin in the magnocellular system and enkephalin in the parvocellular system – are only activated by specific stimuli. Osmotic stimulation only activates dynorphin mRNA and stress only enkephalin mRNA. These two opioid peptides play major roles in both magnocellular and parvocellular systems of the mammalian hypothalamus.

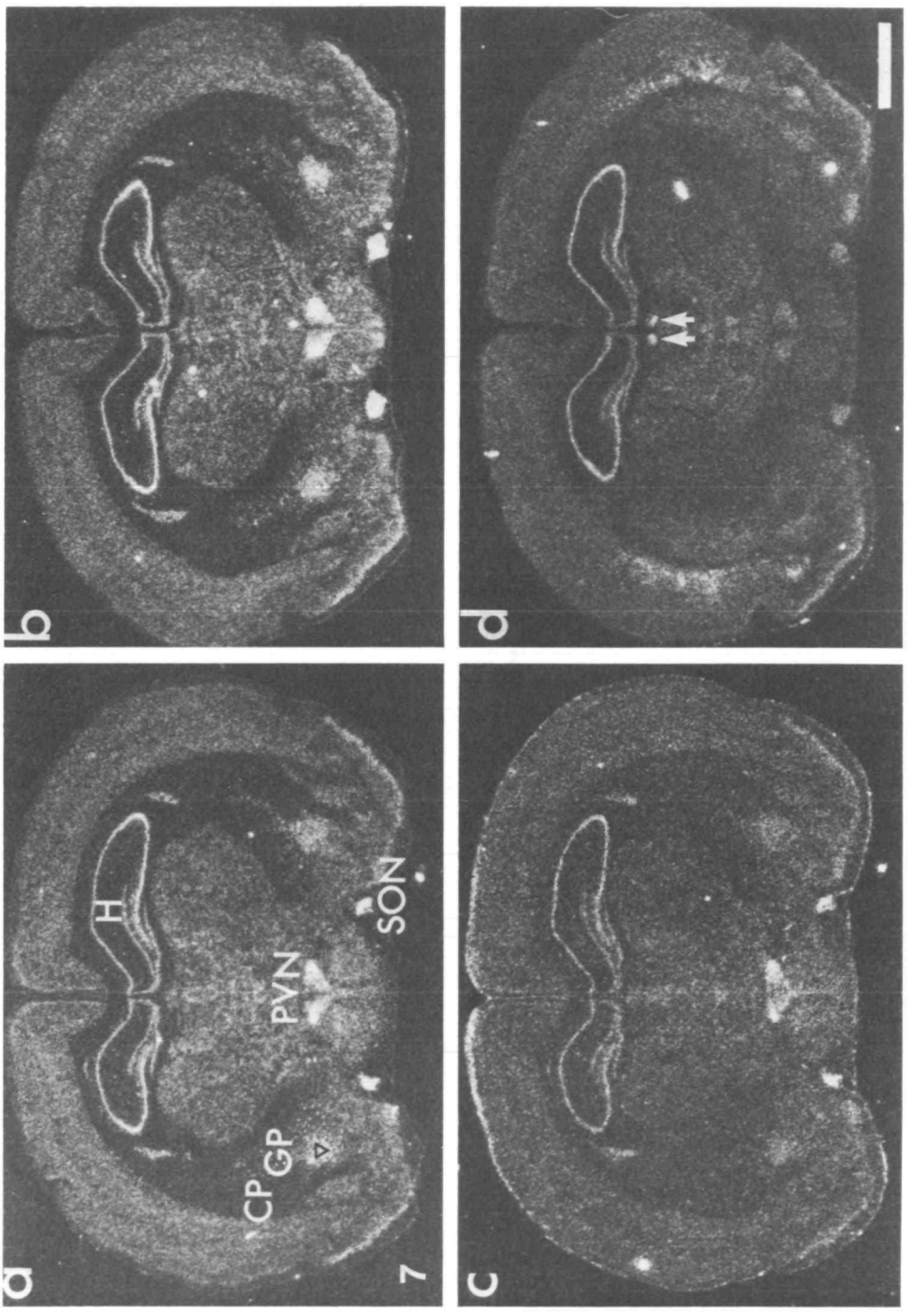
Other roles of *in situ* hybridization histochemical analysis in the magnocellular system

In the previous two sections of this chapter I have illustrated how measurement of the mRNA coding for neuroendocrine peptides which are secreted from a cell can provide information about the expression of their respective genes and reflects changes in biosynthesis and secretion. In addition the technique of quantitative *in situ* hybridization histochemical analysis not only provides high sensitivity but also is unique in identifying individual cells or groups of cells that express the particular gene. There is no reason why similar analyses cannot be applied to the study of regulation of other constituents of cellular metabolism. Two areas of particular interest to the neuroendocrinologist are receptor regulation and the components of the transduction mechanisms which link receptor activation with the appropriate effector mechanism.

Magnocellular acetylcholine-related receptor

Acetylcholine-mediated stimulation of AVP was first demonstrated by Mary Pickford (1939). Injection of acetylcholine into a water-loaded atropinized dog resulted in a marked antidiuresis which could be prevented by prior neurohypophysectomy. The site of action is uncertain. Intracerebroventricular carbachol

Fig. 7. Dark-field autoradiogram of *in situ* hybridization histochemical analysis of cells containing $G_{S\alpha}$ (a,b), $G_{I\alpha}$ (c) and $G_{O\alpha}$ (d) mRNAs. Note increase in signal (silver grains) in the paraventricular (PVN) and supraoptic (SON) nuclei from a salt-loaded (b) compared with a control (a) rat. H, hippocampus; CP, caudate-putamen; GP, globus pallidus; triangle, central nucleus of the amygdala; arrows, medial habenula. Scale bar, 2mm. (From Young, Shepard & Burch, 1987.)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evokes AVP release (Bissett & Chowdrey, 1984; Iitake *et al.* 1986), acetylcholine directly stimulates the electrical activity of SON magnocellular cells (Barker, Crayton & Nicoll, 1971; Sakai, Marks, George & Koestner, 1974) and α-bungarotoxin binds to the magnocellular PVN and SON (Michels, Meeker & Hayward , 1986), although nicotinic receptor activation in the SON does not seem to be through the α-bungarotoxin binding site (Bourque & Brown, 1987).

The involvement of cholinergic neurones in the magnocellular response to osmotic stimuli *in vivo* is suggested by the observation that choline acetyltransferase is found in the SON and PVN (Brownstein, Kobayashi, Palkovits & Saavedra, 1975) and actually falls after 2 weeks of osmotic stimulation (Meyer & Helke, 1981). The reason for this is unclear.

We have approached this problem by making a probe to part of the exon sequence of the rat nicotinic receptor. This probe hybridized to the magnocellular cells in the SON and the PVN (W. S. Young, N. J. Buckley & S. L. Lightman, in preparation). Within 24 h of replacing normal drinking water with 2 % saline there was a significant increase to 242 % of basal levels in the SON and 205 % in the PVN of the amount of probe which hybridized to these nuclei. The increased level of mRNA continued as long as the rats were maintained on 2 % saline (up to 12 days). These data suggest that cholinergic receptors on the magnocellular cells themselves are involved in the osmotic stimulation of vasopressin secretion. Whether the changes in mRNA levels represent increased receptor turnover or up-regulation with greater numbers of cholinergic receptors being expressed needs further investigation with appropriate radiolabelled nicotinic receptor ligands.

Magnocellular G protein and protein kinase C

The intracellular mechanisms underlying the activation of magnocellular neurones by osmotic stimulation or by specific neurotransmitters is unknown. Cell surface receptors may activate the heterotrimer guanine-nucleotide-binding proteins which regulate adenylate cyclase and cyclic AMP (Gillman, 1984; Spiegel, 1987). Although vasopressin and oxytocin do not appear to contain the consensus cyclic-AMP-responsive element sequences, both the CRF (Thompson, Seasholtz & Herbert, 1987) and tyrosine hydroxylase (Lewis, Harrington & Chikaraishi, 1987) genes which are also found in the PVN do contain this sequence. The family of protein kinase C genes is also important in cell activation by hormones and neurotransmitters (Nishizuka, 1986).

Probes complementary to sequences of the α subunits of the G_S, G_I and G_O guanine-nucleotide-binding proteins (Young, Shepard & Burch, 1987) and sequences from three clones isolated from a brain cDNA library by Knopf *et al.* (1986) designated PKC-I, -II and -III have been hybridized with sections of the SON and PVN (Brandt, Niedel, Bell & Young, 1987). Probes to $G_{S\alpha}$, $G_{I\alpha}$ and to a lesser extent $G_{O\alpha}$ all hybridized to the SON and magnocellular area of the PVN, and osmotic stimulation resulted in a significant increase in the amount of both $G_{S\alpha}$ and $G_{I\alpha}$ (Fig. 7). In an interesting extension of their study Young *et al.* (1987) also demonstrated that this increase in G proteins was associated with higher cyclic

AMP levels in the SON, increased cholera-toxin-stimulated adenylate cyclase and an increase in the amount of $G_{S\alpha}$ that was [^{32}P]ADP-ribosylated. In contrast, although PKC transcripts were seen in other areas of the central nervous system, which generally agreed with autoradiographic binding studies using ^{3}[H]phorbol-12,13-dibutyrate (Worley, Baraban & Snyder, 1986), no PKC hybridization was seen in the SON or PVN after 2 months' exposure to emulsion.

These results suggest that, whereas these particular protein kinase C sequences do not play a major role in basal magnocellular activity, G proteins play a significant role in the regulation of the response of these cells to an osmotic stimulus. It would be of considerable interest to know whether the associated changes in cyclic AMP levels play a major role in the increased transcription of the genes which control the biosynthesis of vasopressin, oxytocin and dynorphin (and CRF and tyrosine hydroxylase) which I discussed earlier in this chapter.

Conclusions

The hypothalamus is of pivotal importance in the control of the neuroendocrine system. Studies employing electrophysiology, immunocytochemistry, receptor autoradiography and radioimmunoassay have provided detailed insights into the mechanisms controlling hypothalamic function. Over the last few years molecular biologists have cloned many neurohormones and neurohormone receptors and knowledge of their nucleotide sequence has allowed the development of techniques to localize and measure individual mRNAs. Quantitative hybridization of transcribed mRNA is now proving to be particularly valuable in *in vivo* studies designed to examine the regulation of neuroendocrine neurones in the hypothalamus. In addition to the methods of solution and dot–blot hybridization, quantitative analysis of *in situ* hybridization histochemical studies has proved to be a particularly powerful technique, combining high sensitivity with cellular resolution. Although the most obvious application of this technique is for the study of the neurohormone gene expression, it can also be utilized for studies of receptors and of cellular constituents responsible for transducing receptor activation to the appropriate cellular response. This chapter has concentrated on the application of these techniques to hypothalamic function, but there is no reason why they should not also be applied to any site in the body which responds to its local chemical environment.

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