J. exp. Biol. 139, 67–79 (1988)
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PITUICYTES, GLIA AND CONTROL OF TERMINAL SECRETION

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Summary

Once thought to be hormone-synthesizing cells, the pituicytes are now known to be the resident astroglia of the neurohypophysis (also referred to here as the posterior pituitary). Early investigators interpreted light microscopic observations as demonstrating pituicyte secretion, since pituicytes appeared to contain neurosecretory material when hormone demand was low and not when it was increased. Ultrastructural studies have shown that pituicytes actually engulf or completely surround neurosecretory axons and axonal endings under basal conditions, and release these neural processes when conditions require increased hormone output. Thus, the pituicytes appeared to the early workers to contain and release hormone when they actually contained and released axons and terminals in which the hormone was, in fact, contained. Dynamic interactions of pituicytes with various of the other elements in the gland have also been demonstrated. When hormone demand is low, the pituicytes not only engulf the neurosecretory processes but also interpose their own processes between the secretory endings and the basal lamina. Since any hormone that is secreted must pass through the basal lamina and into the perivascular spaces in order to enter the fenestrated capillaries, pituicyte interpositions form physical, and perhaps chemical, barriers to hormone entering the circulation. Increasing hormone demand results in retraction of pituicyte processes from the basal lamina, permitting increased neural contact. Studies of isolated neurohypophysis and of cultured adult rat pituicytes have shown that these glia undergo appropriate morphological changes in response to osmotic stimuli or to receptor-mediated activation of adenylate cyclase. Both these events are thought to be effectors of the alterations seen *in vivo*. Some possible mechanisms by which pituicytes may participate in the control of secretory events are discussed.

Introduction

Mammalian neurosecretion has perhaps been best and most thoroughly studied in the magnocellular hypothalamo-neurohypophyseal system of the rat. Singular advantages qualify this as a model system. For example, studies can be made of the

Key words: astrocytes, neurohypophysis, neuronal/glia interaction, plasticity.

effects of neural inputs to, as well as of blood-borne factors on, the cellular elements in the hypothalamic portions of the system. At the same time, the events accompanying changes in secretory activity at the neurohypophyseal terminals of those same hypothalamic neurones can be observed. After a brief discussion of the organization of the magnocellular hypothalamo-neurohypophyseal system as a whole, this review will focus upon secretion-related aspects of the neurosecretory terminals and their associated structures in the posterior pituitary (also referred to here as either the neurohypophysis or the neural lobe).

Secretion of the neurohormones oxytocin and vasopressin from the terminals located in the posterior pituitary occurs in response to activation of the magnocellular neurones that reside in the anterior hypothalamus. Although many of these neurones (nearly half) are located in so-called 'internuclear' or 'accessory' groups (Fisher, Price, Burford & Lederis, 1979), most of the information available on the structure and function of this system comes from the two largest condensations of cells, the paraventricular and supraoptic nuclei. In the rat, it has been shown that many hormone-containing axons of magnocellular neurones give rise to collaterals which appear to terminate in hypothalamic areas adjacent to the paraventricular (Hatton, Cobbett & Salm, 1985) and supraoptic nuclei (Mason, Ho & Hatton, 1984). The presence of such collaterals, and stimulated oxytocin and vasopressin secretion into their terminal areas (Mason *et al.* 1986), suggests that information concerning the secretory events that are to take place in the posterior pituitary is delivered first to selected hypothalamic neurones in the form of peptidergic signals. Feedback or local circuits seem to be possibilities here, since activation of these collaterals generally excites neurones in the areas to which they project (Hatton *et al.* 1985; Mason *et al.* 1984).

As they traverse caudally and medially, axons from the magnocellular neurones that are destined to terminate in the posterior pituitary collect into the hypothalamo-neurohypophyseal tract. This tract courses through the internal zone of the median eminence and the infundibulum to end in the neural lobe of the pituitary.

Hypothalamic magnocellular neurones are known to be activated by a variety of stimulus conditions, such as haemorrhage, increased plasma osmotic pressure, the events leading up to parturition and the suckling of pups during lactation. All but the last of these stimulus complexes result in secretion of both oxytocin and vasopressin from the neurohypophysis, suckling being a stimulus for the selective release of oxytocin into the blood (for a review see Poulain & Wakerley, 1982). The relative contributions of synaptic inputs and blood-borne factors in stimulating hormone release under these various conditions are not known with any degree of certainty. In part, this is because the synaptic inputs to the magnocellular neurones are still under investigation. Research to date suggests that there are synaptic influences from a variety of sources at both the hypothalamic and neurohypophyseal levels of this system (Baumgarten, Bjorkland, Holstein & Nobin, 1972; Buijs, van Vulpen & Geffard, 1987; Morris, 1983; Renaud, 1987; Swanson, 1986; van Leeuwen, Pool & Sluiter, 1983).

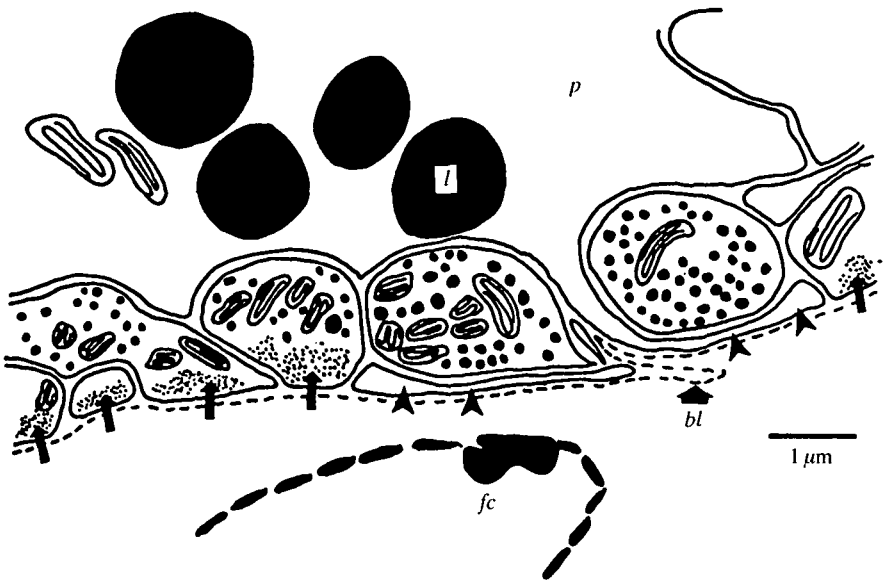

Fig. 1. Diagrammatic representation of the neurovascular contact zone in rat neurohypophysis. The basal lamina (*bl*), represented by a dashed line, is occupied either by pituicyte processes (arrowheads) or by neurosecretory terminals (arrows) containing microvesicles (stippling). Other neurosecretory granule-containing endings (indicated by large filled circles in the endings) are separated from the *bl* by pituicyte processes and do not contain microvesicles. Lipid inclusions (*l*) are shown in the pituicyte (*p*) cytoplasm; *fc*, fenestrated capillary.

Organization of the neurohypophysis

Dominating the neurohypophyseal landscape are the neurosecretory axons, axonal swellings called Herring bodies, and axonal endings of the magnocellular neurones, all of which contain hormone in dense-core vesicles. In addition to the tens of thousands of these structures, the neural lobe contains non-neurosecretory axons, basal lamina, capillaries that are fenestrated (which place the other neurohypophyseal elements outside the blood–brain barrier) and glial cells called pituicytes. Astrocytic in nature (Salm, Hatton & Nilaver, 1982; Suess & Pliška, 1981), the pituicytes are the only cell bodies of neural origin in the posterior pituitary. In the rat, these fairly large cells (nuclear diameter of approximately 5 μm) contain electron-dense bodies (lipid inclusions) which serve as a convenient observable marker for pituicytes (see Wittkowski, 1986, for an extensive review of this cell type).

The general relationships among many of the elements that constitute the neurohypophysis are shown diagrammatically in Fig. 1 and in the electron micrograph of Fig. 2. It can be seen that for the substances released from the terminals to enter the fenestrated capillaries, they must first traverse the basal lamina and the perivascular space. Generally, it is believed that only those axonal endings that both abut the basal lamina and contain microvesicles are actively

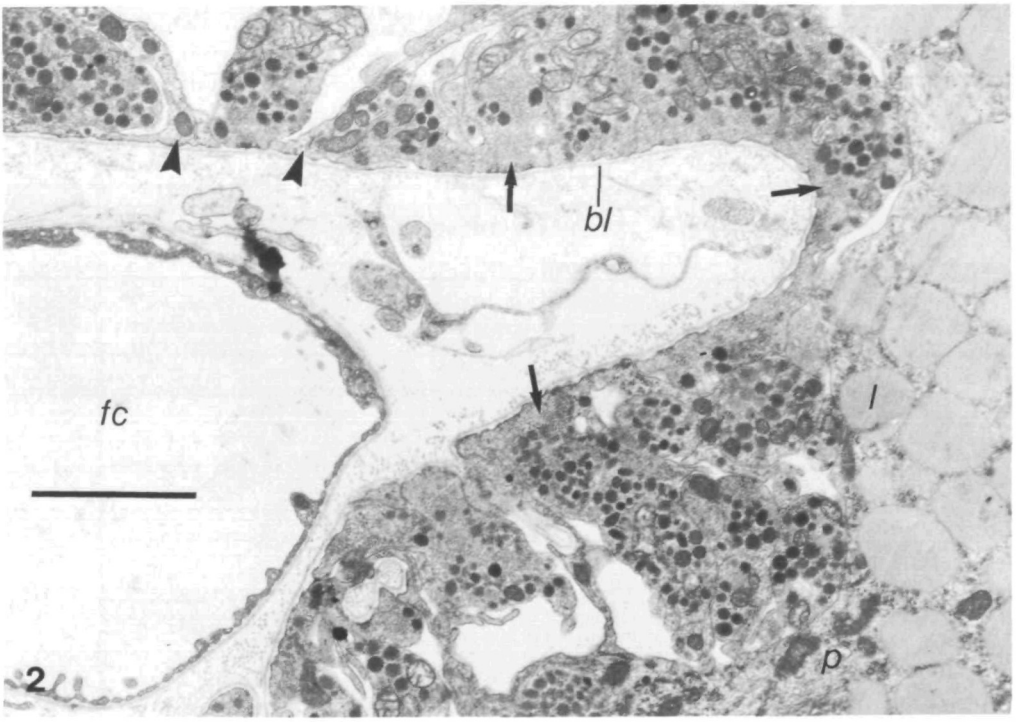

Fig. 2. Electron micrograph of neurovascular contact zone in rat neurohypophysis showing pituicyte (*p*) enclosing neurosecretory axon profiles. The pituicyte lipid inclusions (*l*) can be seen. Both terminals (arrows) and pituicyte cytoplasm (arrowheads) abut the basal lamina (*bl*) separating the neural and glial elements from the perivascular space and a fenestrated capillary (*fc*). Scale bar, 2 μm.

secreting (Morris & Nordmann, 1980). Those that are separated from the basal lamina by pituicyte processes or by other axonal processes usually contain large dense-core vesicles and not microvesicles. There has been a recent suggestion, based on preliminary ultrastructural evidence, that a small amount of exocytotic release of hormone may occur at sites remote from the basal lamina (Morris, 1987).

Early investigations of neural lobe structure and function led to the hypothesis that the pituicytes actually synthesized neurohypophyseal hormones and released them in response to neural signals from the hypothalamus (Fisher, Ingram & Ranson, 1938; Gersh, 1939). This view was largely based on light microscopic observations of the pituitary, and apparent pituicyte accumulation of hormone in well-hydrated animals with subsequent depletion upon dehydration. Bargmann & Scharrer (1951), however, corrected this view by demonstrating that the hormone was actually synthesized in the hypothalamic neurones and reached the neural lobe by axoplasmic transport. Even the accumulations of neural lobe hormone during states of low demand for its release, were found to occur in the neuronal swellings and not in the pituicytes *per se*. Was there, then, any role in secretion for

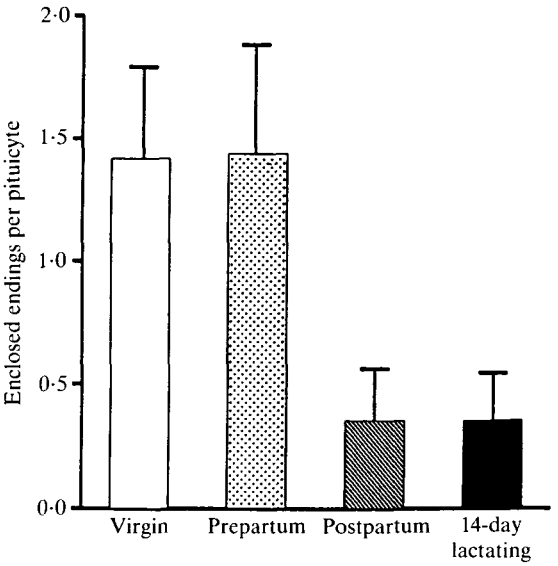

Fig. 3. Neurosecretory axonal enclosure by pituicytes in the neurohypophysis of female rats at four different stages of the reproductive cycle (means and S.E.). Virgin rats were unselected for stages of the oestrus cycle: Prepartum animals on the last day of gestation; 2–20 h after pup delivery; lactating, mothers nursing 6–10 pups for 14 days. (Data from Tweedle & Hatton, 1982.)

the pituicytes? Pituicyte participation in the control of posterior pituitary hormone release from the neural terminals was recognized as a possibility as early as the late 1950s by Leveque & Small (1959). These workers hypothesized that pituicytes secreted a substance which caused 'the release of or the separation of the hormones from a carrier substance' (p. 913), their reasoning being based partly on others' observations that pituicytes contained releasable granules. Ultrastructural studies later revealed that the granules seen in the pituicytes under conditions of low hormone demand were actually inside neurosecretory axons that were themselves being surrounded or engulfed by pituicyte cytoplasm (Dreifuss, Sandri, Akert & Moor, 1975; Tweedle & Hatton, 1980*a*). Although it cannot be ruled out that pituicytes liberate neuroactive material, they do not synthesize dense-core vesicles and so are unlikely to affect hormone release exactly as proposed by Leveque & Small (1959). Evidence continues to mount, however, in favour of pituicytes as influential factors, both subtle and profound, in the control of neurohypophyseal secretion. The evidence accumulated presents a picture of dynamic interactions among the elements of the neurohypophysis as physiological conditions and, thus, demand for hormone output changes.

Under basal conditions of little or no hormone demand, the pituicytes occupy a relatively greater and the neural elements a relatively lesser extent of the basal lamina (Tweedle & Hatton, 1987; Wittkowski & Brinkmann, 1974). Also, under such conditions, the pituicytes are likely to be found surrounding or engulfing

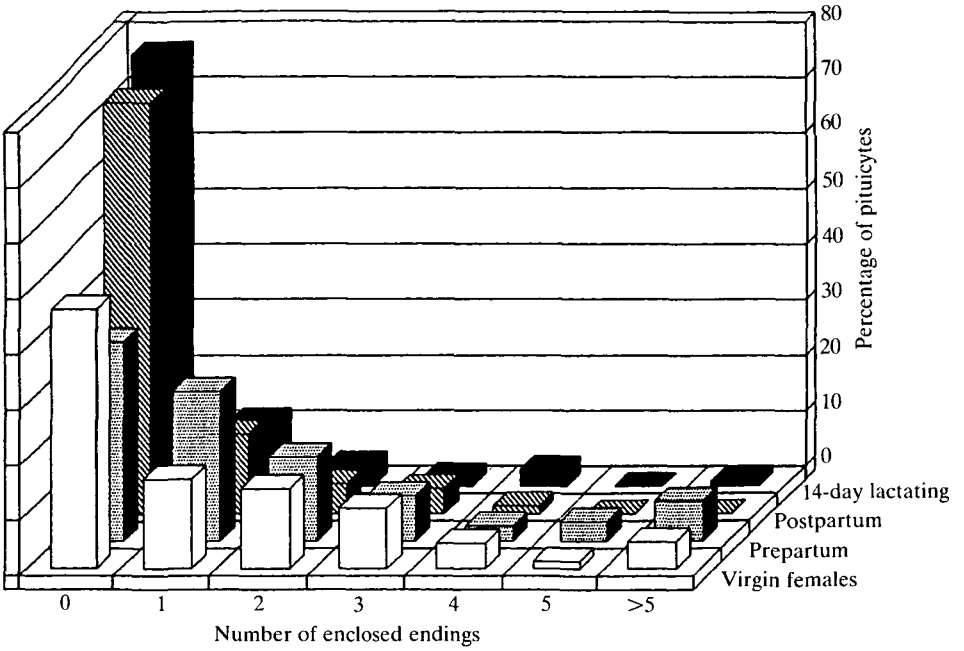

Fig. 4. Percentage of pituicytes containing from zero to five and more than five completely enclosed axonal profiles. Treatment groups are the same as those in Fig. 3. Note that the postpartum and 14-day lactating animals had the largest percentages of pituicytes containing zero and the smallest percentages containing five or more axonal endings. (Data from Tweedle & Hatton, 1982.)

more neurosecretory axonal processes and endings (see Fig. 3). Individual pituicytes are also observed to enclose a larger number of axonal processes under basal than under stimulated conditions (see Fig. 4). Two other changes appear to occur with stimulation: one involves the number of lipid inclusions in the pituicytes and the other is the number, and perhaps the nature, of the axoglial synaptoid contacts onto pituicytes.

Recently, it has been found that the neurosecretory axons make *en passant* contacts with the basal lamina (Tweedle, Smithson & Hatton, 1988). This finding helps to explain the large discrepancy between the numbers of secretory magnocellular neurones in the hypothalamus and the many-fold greater numbers of terminals at the neurovascular contact zone in the neurohypophysis. It also may have implications for how a relatively small number of pituicytes might exert a significant degree of control over secretion at the neurohypophyseal level.

Secretion-related changes

Neurohypophyseal ultrastructure changes markedly in association with stimulus-evoked hormone release. This is the case whether the stimulus is acutely or chronically applied, although there are some differences in response to these two classes of stimuli. Common to all stimulus conditions so far observed which

Table 1. *Changes in pituicyte–neurosecretory terminal relationships under various conditions, differing in hormone demand (means ± S.E.)*

Treatment groups (*N*)	% Neural contact at the basal lamina	Length of terminals (μm)	No. of terminals per 100 μm basal lamina
Virgin (4)	70·9 ± 3·6	1·46 ± 0·07	42·3 ± 2·3
Prepartum (4)	64·4 ± 3·1	1·22 ± 0·02[a]	45·8 ± 1·1
Postpartum (5)	76·2 ± 2·0[b]	1·40 ± 0·09[b]	44·1 ± 1·2
Lactating (4)	83·9 ± 1·5[a,b]	1·53 ± 0·04	55·0 ± 0·8[a,b,c]
Postweaning (4)	79·0 ± 1·2[a,b]	1·52 ± 0·04	46·6 ± 1·6

[a] $P < 0·02$ compared with control; [b] $P < 0·02$ compared with prepartum; [c] $P < 0·02$ compared with postpartum.

Data from Tweedle & Hatton (1987).

increase hormone demand is a decrease in the number of secretory axons enclosed by pituicytes (Tweedle & Hatton, 1980*b*, 1982, 1987). This decreased engulfment is presumably due to release of the axons by the pituicytes (see Figs 3, 4). Stimuli evoking hormone secretion also characteristically result in a decrease in the percentage of pituicyte membrane in contact with the basal lamina, with a proportionate increase in neurosecretory axonal membrane making such contact (Tweedle & Hatton, 1987; Tweedle, Modney & Hatton, 1988; Wittkowski & Brinkmann, 1974). It can be seen from the data presented in the second column of Table 1 that there is a profound increase in the percentage of neural contact at the basal lamina at parturition. This increased contact is maintained during lactation and for at least the first 10 days of the post-weaning period. Equally striking is the release of the axons which also occurred at the time of parturition (see Fig. 3). It appears from the available evidence that these changes are, in large part at least, produced by pituicyte retraction from the basal lamina and active release of axons by pituicytes. There are, however, no data to rule out the possibility that neurosecretory terminals also actively 'seek' positions closer to the neurovascular contact zone.

Of the numerous synaptoid contacts that have been described between axons and pituicytes, only two types have been characterized immunocytochemically. One type contained opioid peptide-like immunoreactivity (van Leeuwen *et al.* 1983) and the other type immunostained positively for gamma-aminobutyric acid (GABA) (Buijs *et al.* 1987; Vincent, Hökfelt & Wu, 1982). It is not unlikely that other such contacts will be found to contain catecholamines or other neurotransmitters. Water deprivation of 72 h duration has been reported to increase the numbers of (unidentified) synaptoid contacts onto pituicytes (Wittkowski & Brinkmann, 1974). It remains to be determined whether such changes in the synaptoid contacts would occur with milder stimuli, e.g. 8 or 12 h of deprivation, which have been found to produce changes in some of the other relationships between axonal endings and pituicytes (Tweedle & Hatton, 1980*b*). Finding

changes with stimuli in this more physiological range would seem to be important in efforts to define a possible functional role for increased synaptoid contacts in the neural lobe. At present, one can only speculate that any neuroactive substances released at these axoglial synaptoid contacts affect pituicyte ion channels, as they do in neurones, and/or perhaps pituicyte morphology.

A rather confusing pattern of results has emerged with respect to the secretion-related changes that occur in the number of liposomes in pituicyte perakarya. Mild deprivation of only 4 h was found to produce a two-fold increase in the numbers of liposomes per 100 μm^2 of pituicyte cytoplasm (Tweedle & Hatton, 1980*b*). Longer durations of deprivation up to 24 h or rehydration after 24 h of deprivation yielded numbers of liposomes that were intermediate between the basal values and the early, 4 h, peak numbers. These results suggested that increases in liposome numbers within the pituicytes were associated with or triggered by the onset of secretion. This is also a time when pituicytes release engulfed axons and may, therefore, be recycling their own membrane (Gregory, Park & Tweedle, 1982). Thus, it may be that this process is reflected in increased lipid inclusions. Other workers, however, have found increases in numbers of liposomes after 36 h of water deprivation (H. F. Reinhardt, L. C. Henning & H. P. Rohr, 1969, cited in Wittkowski, 1986). Nordmann (1985) observed large increases in liposome numbers upon rehydration following 4 days of dehydration. These increases were accompanied by increases in pituicyte volume; however, no clear idea of the functional significance of these changes has emerged.

Acute and chronic stimulation of hormone release in this system have different effects on the sizes and numbers of neurosecretory terminals contacting the basal lamina. Short-term activation of the system, such as occurs with mild water deprivation, during parturition (Tweedle & Hatton, 1987), or with acutely elevated levels of testosterone (Tweedle *et al.* 1988), results in enlarged terminal contacts with the basal lamina. In contrast, lactation and suckling of pups (Tweedle & Hatton, 1987) or prolonged elevation (30 days) of testosterone (Tweedle *et al.* 1988), produces increases in the number of terminal contacts with the basal lamina without any change in the size of the terminals. An example of this differential effect is given in the data presented in the third and fourth columns of Table 1. There is a curious significant decrease in the size of the neurosecretory terminals by the time of, but just preceding, parturition. A few hours later, however, the postpartum animal's terminals have increased in size back to control values. Note that the peripartum events took place with no changes in number of terminals per unit length of the basal lamina. In contrast, the chronic stimulation provided by 14 days of lactation resulted in significantly more terminals, but of normal size, along the basal lamina (fourth column). It appears, therefore, that the adaptations made by the system during the initial phases of activation are further modified if and when stimulation becomes chronic. This is compatible with what is observed at the hypothalamic level of this system under acute *vs* chronic stimulation. For instance, short-term activation produces glial retraction fr[illegible] between the dendrites and the cell bodies of supraoptic neurones, whereas long-

term elevations in hormone demand may cause glial proliferation (Murray, 1968; Paterson & Leblond, 1977) and induce the formation of new synapses (see Hatton, 1985; 1988, for reviews).

Another factor that is differentially affected by acute or short-term, as opposed to chronic or protracted, elevations in hormone demand is the rapidity with which the induced changes are reversed after demand for hormone is returned to normal. For example, the decrease in the number of secretory axonal processes enclosed by pituicytes that is produced by 24 h of water deprivation is completely reversed by 24 h of free access to tap water (Tweedle & Hatton, 1980*b*). However, the number of axonal processes enclosed by pituicytes following 10 days of hypertonic saline drinking does not return to control values even after 2 weeks of rehydration. Five weeks of free access to tap water were sufficient to re-establish the numbers of enclosures seen under basal conditions (Tweedle & Hatton, 1987). Once again, similar effects have been observed in the hypothalamic portions of this system. Perlmutter, Tweedle & Hatton (1985) found that both acute and chronic dehydration resulted in increased bundling of supraoptic nucleus dendrites, whereas rehydration following dehydration reinstated the normal picture of mostly single dendrites separated from their neighbours by astrocyte processes. In the case of acute dehydration, however, a duration of rehydration equivalent to the dehydration interval was sufficient to reverse these effects. Rehydration durations equivalent to the interval of chronic dehydration were insufficient to re-establish control values, although complete reversal of the induced changes did eventually occur. Thus, the response by far outlasts the stimulus. An intriguing question might be: does the system develop some kind of memory, particularly in response to chronically applied stimuli, such that subsequent applications would produce more rapidly appearing or more profound changes? Complete answers to this question await experimental data.

Pituicyte actions *in vitro*

Several effects similar to those seen *in vivo* have also been observed *in vitro*, either in the isolated neural lobe or in cultured pituicytes. For instance, manipulations of the osmotic pressure of the medium bathing the neurointermediate lobe *in vitro* have been shown to result in changes similar to those produced by dehydration *in vivo* (Perlmutter, Hatton & Tweedle, 1984). Increasing the osmolality of the medium from 290 to 310 or 340 mosmol kg^{-1} for 2 h caused a progressive increase in neural contact with the basal lamina and a decrease in the numbers of engulfed axonal processes per pituicyte. Since basal release of hormone is not affected by this manipulation, neuropeptides are unlikely to be responsible for these effects. Thus, pituicytes appear to be stimulated directly by and respond appropriately to osmotic changes, such as those occurring in the blood *in vivo* during dehydration. This is not to deny a role for neural input to pituicytes in the intact animal.

An opportunity to study these mechanisms further was made available by the demonstration that adult rat pituicytes could be successfully maintained in culture (Bicknell, Leendertz & Worley, 1983; see also Hatton, Perlmutter, Salm & Tweedle, 1984). Pituicytes that have been maintained in culture medium for 10 days or so assume a flattened, amorphous shape. This morphology is similar to that observed ultrastructurally *in vivo* under basal conditions. It has long been known that astrocytes cultured from foetal brains respond to applications of certain substances (e.g. hormones, cyclic nucleotides) with changes in their morphology (for a review see van Calker & Hamprecht, 1980). It is also known that such astrocytes possess a variety of receptors, e.g. alpha- and beta-adrenergic, and H_1- and H_2-histaminergic receptors (Hösli & Hösli, 1982, 1984; Hösli *et al.* 1982). When the flattened, amorphous adult rat pituicytes in culture are treated with low concentrations (10 nmol l^{-1}) of dibutyryl cyclic AMP, they rapidly become stellate (i.e. astrocyte-like). It is this morphology that is seen *in vivo* under conditions of enhanced hormone release. Concentrations of 10–100 nmol l^{-1} of the beta-adrenergic agonist isoproterenol also produced pituicyte stellation, a response that was either reversed or blocked by the antagonist propranolol (R. J. Bicknell, G. I. Hatton, S. Luckman & W. T. Mason, unpublished results). This action of the beta-agonist is likely to be through its ability to activate adenylate cyclase and cause the accumulation of cyclic AMP. *In vivo* pituicytes probably receive noradrenergic signals, since noradrenaline is released in states of increased hormone demand (Holzbauer *et al.* 1980). Similar drug manipulations have now been made using the isolated neurointermediate lobe *in vitro* (I. Suarez, K. G. Smithson & G. I. Hatton, unpublished results). Quantitative ultrastructural analyses were made of neurohypophyses that had been maintained in a defined medium and given a 15-min pulse of either the same control medium or isoproterenol (10 or 100 nmol l^{-1}). The percentage of basal lamina occupied by neural, as opposed to pituicyte, membrane was significantly increased in the pituitaries treated with the beta-agonist. This effect was similar to those seen with osmotic stimulation *in vivo* (Tweedle & Hatton, 1987; Wittkowski & Brinkmann, 1974). Taken together, these findings suggest that osmotic stimuli and neurotransmitters may act in concert to produce pituicyte changes that are appropriate to the physiological condition of the animal.

Pituicytes and secretion

Is it, then, reasonable to suppose that pituicytes participate in the control of secretion from the neurohypophysis and, if so, what may be some of the mechanisms of this control? First, given all the evidence for dynamic interactions at functionally meaningful times between the pituicytes and other elements of the neural lobe, it seems likely that the pituicytes are playing much more than a simple passive role in controlling neurohypophyseal secretion. The presence of pituicyte processes interposed between the neurosecretory terminal membrane and the basal lamina clearly constitutes a potential physical and perhaps chemical barrier

to secreted peptide entering the circulation. By occupying a relatively greater extent of the basal lamina during periods of low hormone demand, the pituicytes may, to a greater or lesser extent, restrict the amount of hormone that is free to enter the perivascular space. Another mechanism by which the pituicytes may at least partially control secretion is by restricting the periterminal extracellular space of the engulfed axonal endings. Such restriction would serve to amplify any autoreceptor negative feedback that may exist. Recent evidence for the existence of vasopressin receptors on neural lobe terminals (Bunn, Hanley & Wilkin, 1986) supports this hypothesis. It is also possible that the pituicytes that surround axons and/or engulf terminals could control secretion by blocking the propagation of action potentials, thereby disallowing both the depolarization of the terminal and the resulting peptide release. This could be accomplished by altering the ionic microenvironment around the neural membrane or by release of inhibitory transmitters, such as GABA. Ionic species likely to be involved here are K^+ and Ca^{2+}. Since pituicytes themselves apparently receive GABAergic inputs (Buijs *et al.* 1987) and astrocytes respond to GABA by opening chloride channels (Kettenmann, Backus & Schachner, 1987), Cl^- may also be involved. If pituicytes are able both to take up and to release GABA, as earlier data suggested (Minchin & Nordmann, 1975), they may also directly influence axonal membrane chloride conductances. Of possible importance here may be our recent finding that individual neurosecretory axons make multiple *en passant* contacts with the basal lamina. This being the case, a single pituicyte that surrounds several axons (see Fig. 4) would be able to inhibit secretion from perhaps tens to hundreds of terminals.

Much work remains to be done to determine which of these mechanisms are important and under what conditions they are operative. It may be that different blood-borne and neural inputs to the pituicytes are associated with different control mechanisms. It is also likely that there are mechanisms operating in the control of secretory events in the neural lobe for which we have no information. Pituicytes, however, are likely to be participants in these as yet undisclosed mechanisms, as they appear to be in those about which we are beginning to gain some understanding.

This review and some of the research summarized in it was supported by NIH grants NS09140 and NS16942. Thanks are due B. K. Modney, C. D. Tweedle and M. L. Weiss for helpful suggestions on an earlier draft of the manuscript, and to K. Grant, I. Taubitz and J. Harper for technical and typing assistance.

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