Synthesis, Transport, and Release of Posterior Pituitary Hormones

Michael J. Brownstein, James T. Russell, Harold Gainer

Summary. Vasopressin and oxytocin are made and released by neurons of the hypothalamo-neurohypophysial system. Pulse labeling these neurons with radioactive amino acid indicates that the two hormones and their respective neurophysin carrier proteins are synthesized as parts of separate precursor proteins. The precursors seem to be processed into smaller, biologically active molecules while they are being transported along the axon.

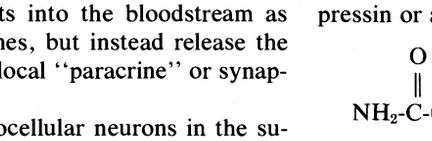
Ever since the discovery of the neurosecretory cell by Ernst Scharrer a half-century ago (*1*), the significance of this type of neuron for nervous system function has intrigued biologists. Neurosecretory cells—cells that have both neural and endocrine properties—are present in nervous systems ranging from those of coelenterates to that of man. In fact, some authors have postulated that the neurosecretory cell may be an evolutionary antecedent of "conventional" neurons which use small molecules such as acetylcholine or the biogenic amines as neurotransmitters (*2*).

Neurosecretory cells represent only one class of peptidergic neuron (that is, neurons that synthesize and secrete peptides). In the last decade a wide variety of peptide-producing neurons has been discovered in the brain and periphery. These neurons do not necessarily secrete their products into the bloodstream as neurohormones, but instead release the peptides for local "paracrine" or synaptic actions.

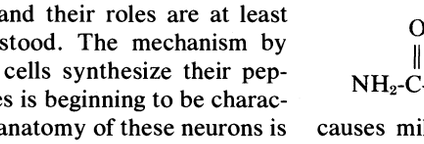
The magnocellular neurons in the supraoptic and paraventricular nuclei are model peptidergic neurons. The peptides secreted by the magnocellular neurons are known, and their roles are at least partly understood. The mechanism by which these cells synthesize their peptide hormones is beginning to be characterized. The anatomy of these neurons is well defined. Some of the neurotransmitters involved in regulating hormone release are known; and finally, the electrophysiological properties of the magnocellular neurons have been the subject of intense investigation. In this article we have tried to illustrate the strengths of the hypothalamo-neurohypophysial system for cell biological studies.

The pituitary gland is divided into three parts—the anterior, intermediate, and posterior lobes (Fig. 1). The posterior lobe or neurohypophysis is richly endowed with neuronal processes; in fact, axons and axon terminals comprise about 42 percent of its total volume (*3*). These processes derive from large neurons in the supraoptic and paraventricular nuclei of the hypothalamus (Fig. 1).

The nerve terminals in the posterior pituitary store peptide hormones and release them into blood vessels. Two hormones in the posterior lobe (*4*)—arginine vasopressin (AVP) and oxytocin (OT)—are made by separate populations or neurons in both the supraoptic and paraventricular nuclei (Fig. 1) (*5*, *6*). Vasopressin or antidiuretic hormone (*7*)



causes the kidney to resorb water. Oxytocin



causes milk ejection. It also stimulates contraction of uterine muscle, and may be involved in parturition. Recently, vasopressin and oxytocin have been reported to be present in axons outside of the hypothalamo-neurohypophysial system. The roles played by the peptides in extrahypothalamic areas are unknown.

Vasopressin and oxytocin are stored in secretory granules or vesicles (*8*) along with their respective "carrier proteins," neurophysins (Fig. 2B) (*9*). The neurophysins have molecular weights of about 10,000 and, like vasopressin and oxytocin, contain a very high percentage of cysteine residues linked by disulfide bridges.

Sachs and Takabatake suggested that vasopressin and its neurophysin were synthesized in the cell body as parts of a common precursor protein (*10*). Subsequently, considerable evidence has been obtained which indirectly supports this hypothesis (*11*). A number of workers have suggested that the putative precursor molecule might be synthesized by a ribosomal mechanism on the rough endoplasmic reticulum, and packaged into secretory granules (via the Golgi apparatus) where it is cleaved (processed) into active peptide products (Fig. 2A). It should be noted that in neurosecretory cells the hormone precursors are transported along axons in granules. We shall present evidence below that significant processing of the prohormones takes place intragranularly. Thus, the granules must contain both the precursor and enzymes responsible for its posttranslational processing. The contents of the granules (hormones, carrier proteins, and residual bits of precursor) appear to be released when the nerve endings are depolarized (*12*). This release involves a calcium-dependent fusion of the granules with the nerve terminal membrane followed by opening of the granules and release of their contents (*13*). This process is called exocytosis.

The neurosecretory neuron, unlike other types of cells that synthesize and secrete hormones, executes a sort of morphological segregation of its protein products that can be exploited by the investigator. Among the myriad of proteins manufactured in the cell body, relatively few are selected for rapid transport down the axon as neurohormones and their precursors are. The newly synthesized molecules that have been rapidly transported to axon terminals in secretory granules can be released by depolarizing the terminals. Thus, after radioactive amino acids are infused in the region of the cell bodies to label the proteins there, two cellular processes can be observed to help distinguish the hormones and their precursors from other

Dr. Brownstein is a member of the Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, Maryland 20205. Drs. Russell and Gainer are members of the Laboratory of Developmental Neurobiology, National Institute of Child Health and Human Development, Bethesda.

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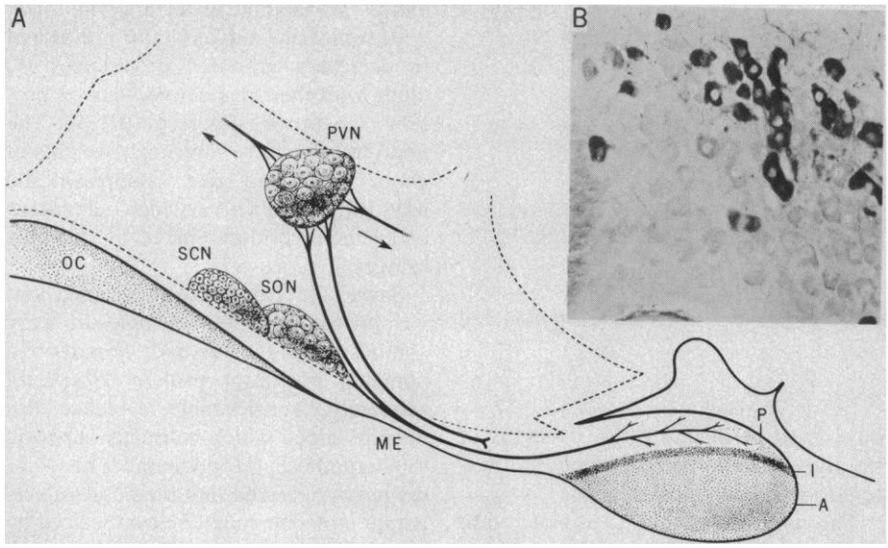

Fig. 1. The hypothalamo-neurohypophysial system. (A) Large (magnocellular) neurons in the supraoptic (*SON*) and paraventricular (*PVN*) nuclei send their axons to the posterior pituitary (*P*) via the median eminence (*ME*). Cells in the paraventricular also project to the median eminence and to extrahypothalamic areas, among them the medial nucleus of the amygdala, the tract and nucleus of the diagonal band, the mesencephalic central gray, the Edinger-Westphal complex, the marginal nucleus of the brachium conjunctivum, the locus coeruleus, the nucleus of the solitary tract, the nucleus intermedium, the dorsal motor nucleus of the vagus, and the intermediolateral column, central gray, and marginal zone of the spinal cord. Two peptide hormones, vasopressin and oxytocin, and their neurophysin carrier proteins are produced by different neurons in the supraoptic and a paraventricular nuclei. Vasopressin is synthesized by small (parvicellular) neurons in the suprachiasmatic nuclei (*SCN*) as well. *I* indicates intermediate lobe and *A* anterior lobe of the pituitary. (B) Neurophysin is visualized in the supraoptic nucleus of Brattleboro rats in some, but not all, magnocellular neurons. The neurophysin-positive neurons in the dorsal pole of the nucleus manufacture oxytocin and its neurophysin. The nonstaining neurons would, in normal rats, make vasopressin and its neurophysin. [From Sokol *et al.* (*6*); courtesy of *Endocrinology*]

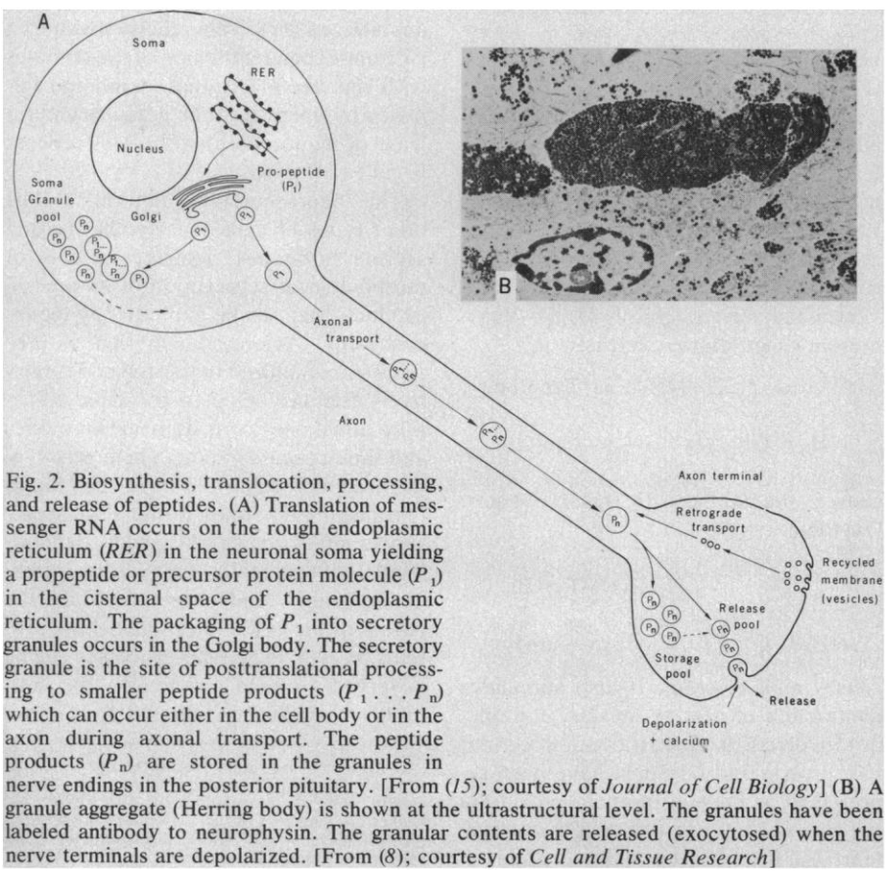

Fig. 2. Biosynthesis, translocation, processing, and release of peptides. (A) Translation of messenger RNA occurs on the rough endoplasmic reticulum (*RER*) in the neuronal soma yielding a propeptide or precursor protein molecule (P_1) in the cisternal space of the endoplasmic reticulum. The packaging of P_1 into secretory granules occurs in the Golgi body. The secretory granule is the site of posttranslational processing to smaller peptide products ($P_1 \ldots P_n$) which can occur either in the cell body or in the axon during axonal transport. The peptide products (P_n) are stored in the granules in nerve endings in the posterior pituitary. [From (*15*); courtesy of *Journal of Cell Biology*] (B) A granule aggregate (Herring body) is shown at the ultrastructural level. The granules have been labeled antibody to neurophysin. The granular contents are released (exocytosed) when the nerve terminals are depolarized. [From (*8*); courtesy of *Cell and Tissue Research*]

labeled molecules: rapid axonal transport (rate greater than 200 millimeters per day) and exocytotic release.

Identification of Neurophysin Precursors

Our experimental approach was based on the model of the peptidergic neuron described above (*14*, *15*). We injected 1 microliter of [^{35}S]cysteine in normal saline just above and lateral to the supraoptic nuclei of ether-anesthetized rats. (Cysteine was chosen for labeling because vasopressin, oxytocin, and the neurophysins are particularly rich in this amino acid.) The rats were allowed to recover from the anesthetic, and were killed by decapitation at various times after injection. Their brains and pituitaries were rapidly removed and frozen, and samples of tissues were obtained for analysis from the supraoptic nucleus (SON) (neuronal perikarya), median eminence (axons), and posterior pituitary (nerve endings) (*16*).

The labeled cysteine was incorporated quickly into proteins in the SON. Labeled proteins first appeared in the median eminence in about 1 hour, and in the posterior pituitary in about 1.5 hours, after injection of the amino acid (Fig. 3). The lag between incorporation of [^{35}S]cysteine into proteins in the SON and appearance of labeled proteins in the median eminence and pituitary is accounted for by axonal transport of the proteins from the SON along the axons. Our estimate of the fast transport rate in this system (> 200 mm/day) was consistent with values reported earlier (*17*). We also found, in agreement with previous reports, that colchicine completely prevented the transport of proteins labeled in the SON to the median eminence and posterior pituitary (Fig. 3).

When the labeled proteins that traveled to and accumulated in the posterior pituitary 12 to 24 hours after [^{35}S]cysteine was injected were subjected to electrophoresis on acid-urea polyacrylamide gels, a simple pattern emerged (*14*). The label was virtually confined to a single peak, which shared several properties with the neurophysins: (i) it was the major cysteine-rich acid-soluble protein transported to the pituitary, (ii) it was highly soluble in alcohol (*18*), (iii) it had a molecular weight of 10,000 to 12,000, (iv) it reacted with three different antibodies to neurophysin, (v) it comigrated on the gel with a Coomassie blue stained band, which specifically decreased in intensity in pituitaries of rats given 2 percent saline to drink (*15*), and (vi) it was released from the pituitary by potassium depolarization

(*15*) as is neurophysin (*19*). The labeled neurophysin-like material could be resolved by means of isoelectric focusing into two separate proteins, with isoelectric points (*p*I's) of 4.6 and 4.8 (Fig. 4C) (*20*). One of these proteins (*p*I 4.8) was absent from the Brattleboro rat (Fig. 4C) (*20*), a strain that has diabetes insipidus because it cannot make vasopressin (and the vasopressin-associated neurophysin). Therefore, we concluded that the *p*I 4.8 protein was the vasopressin-associated neurophysin (Np-AVP) and that the *p*I 4.6 protein was the oxytocin-assocated neurophysin (Np-OT). If these neurophysins came from separate Np-AVP and Np-OT precursors we should be able to distinguish one from the other by comparing normal and Brattleboro rats.

Initially, we looked for the biosynthesis of neurophysin precursors in the SON where the machinery for protein synthesis is located. We found that [^{35}S]cysteine injected into the SON of normal animals was initially incorporated into relatively large proteins that disappeared in time and that appeared to be transformed into labeled proteins with lower molecular weights (*14, 15*). These data were analagous to those that led Steiner and his co-workers to infer the existence of proinsulin (*21*), the insulin precursor.

Soon after [^{35}S]cysteine was injected, the SON contained two major labeled peaks with *p*I's of 6.1 and 5.4 (Fig. 4A) and apparent molecular weights [as determined on sodium dodecyl sulfate (SDS) gels] of 20,000 to 22,000 (*20*). One hour after the injection, two additional peaks appeared with *p*I's of 5.6 and 5.1 and molecular weights of 15,000 to 17,000 (Fig. 4B) (*20*).

The *p*I 6.1, 5.6, 5.4, and 5.1 proteins all reacted with specific antibodies to rat neurophysin (*22*). Thus, we concluded that the *p*I 6.1 and 5.4 proteins were precursors and the *p*I 5.6 and 5.1 proteins were intermediate forms of neurophysin. Recently, Guidice and Chaiken have shown that messenger RNA isolated from the bovine hypothalamus codes for a large neurophysin-containing protein. This protein (or these proteins) are likely to be the *p*I 6.1 and 5.4 precursors along with their signal sequences (*23*).

Although the above posttranslational conversions of precursors to lower molecular weight products occurred in the SON (that is, cell bodies), it was apparent that processing also took place in the axons. Analyses of labeled proteins arriving in the median eminence and the pituitary 1 to 2 hours after [^{35}S]cysteine injections showed that the labeled *p*I 5.4 and 6.1 proteins were present in abundance in the axons, but that with time all the labeled proteins in the axons were converted to neurophysins. Since autoradiographic studies have shown that all the [^{35}S]cysteine-labeled protein transported to the posterior pituitary is within the secretory granules (*24*), we have concluded that a precursor-to-product conversion must be taking place intragranularly during axonal transport of the precursors. As the axonal transport and processing continued, the SON and median eminence were depleted of labeled precursors, which gave rise to the major stored products in the pituitary, the 10,000- to 12,000-dalton neurophysins.

The oxytocin- and vasopressin-related neurophysin precursors were identified by comparing the proteins synthesized in the SON of normal versus Brattleboro rats (Fig. 4, A and B) (*20*). Twenty minutes after injecting cysteine adjacent to the SON of Brattleboro rats only one major labeled protein appeared, the *p*I 5.4 precursor. One hour after injection this precursor gave rise to the *p*I 5.1 intermediate. On the basis of these data, we concluded that the Np-OT was synthesized from the *p*I 5.4 precursor via the *p*I 5.1 intermediate and that the Np-AVP was synthesized from the *p*I 6.1 precursor via the *p*I 5.6 intermediate.

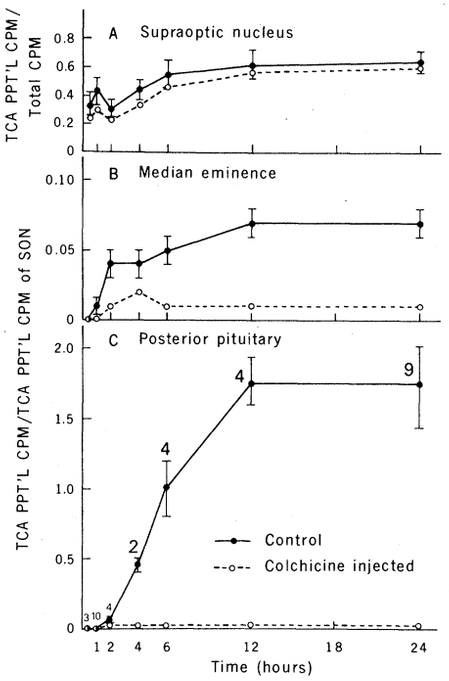

Fig. 3. ^{35}S-Labeled proteins [precipitated by trichloroacetic acid (*TCA PPT'L*)] in the supraoptic nucleus (A), median eminence (B), and posterior pituitary (C) after injection of [^{35}S]cysteine adjacent to the supraoptic nuclei of female rats. Data from control rats (filled circles) and colchicine treated rats (open circles) are plotted. There is considerable labeled protein in the SON 30 minutes after the [^{35}S]cysteine injection, but there is a lag in the appearance of labeled protein in the median eminence (1 hour) and posterior pituitary (2 hours). In the presence of colchicine, protein accumulates in the SON, but is not transported through the median eminence to the pituitary. Data are expressed as means ± standard deviations; the number of experiments at each timepoint is shown in (C). [From (*15*); courtesy of *Journal of Cell Biology*]

Evidence in Favor of the Common Precursor Hypothesis

We attempted to precipitate the *p*I 6.1 neurophysin precursor with antibodies to vasopressin, but failed. We then isolated the *p*I 5.4 and 6.1 peaks and could show some immunoreactivity of the *p*I 6.1 peak by radioimmunoassays for vasopressin but there was no such evidence of immunoreactivity in the *p*I 5.4 peak (*25*). Both peaks reacted with antibody to neurophysin in radioimmunoassays. Although these data were suggestive of and consistent with the common precursor hypothesis, we took a more rigorous approach to the problem.

Analyses of the primary structures of several prohormones have shown that two to three basic amino acids (lysine or arginine) are often neighbors to active peptide sequences. Since trypsin cleaves peptides between the carboxyl group of lysine or arginine and the amino group of any succeeding amino acid, active peptides can often be liberated from their prohormones by incubation of the prohormones with trypsin (*26*). Thus, we reasoned that the labeled precursor of Np-AVP might yield neurophysin-like and vasopressin-like products after limited proteolysis with trypsin. We first developed a set of criteria for identifying the tryptic products. To be considered neurophysin-like, a tryptic product should (i) have a molecular size of 10,000 daltons, (ii) bind to a vasopressin-Sepharose affinity column at *p*H 5.5 and be eluted at more acid or basic *p*H's [that is, show characteristic binding to its natural ligand (*26*)], and (iii) have a *p*I of about 4.6 to 4.8. To be identified as being oxytocin- or vasopressin-like, peptides liberated from the 20,000-dalton precursor should (i) be around 1000 daltons, (ii) bind to a neurophysin-Sepharose affinity column in a *p*H-dependent manner (*27*), and (iii) react with antibodies generated against the nonapeptides. Since tryptic cleavage is likely only to be the first step in prohormone processing, we did not expect the tryptic peptides to be identical to vasopressin and oxytocin. In the absence of a trimming enzyme like

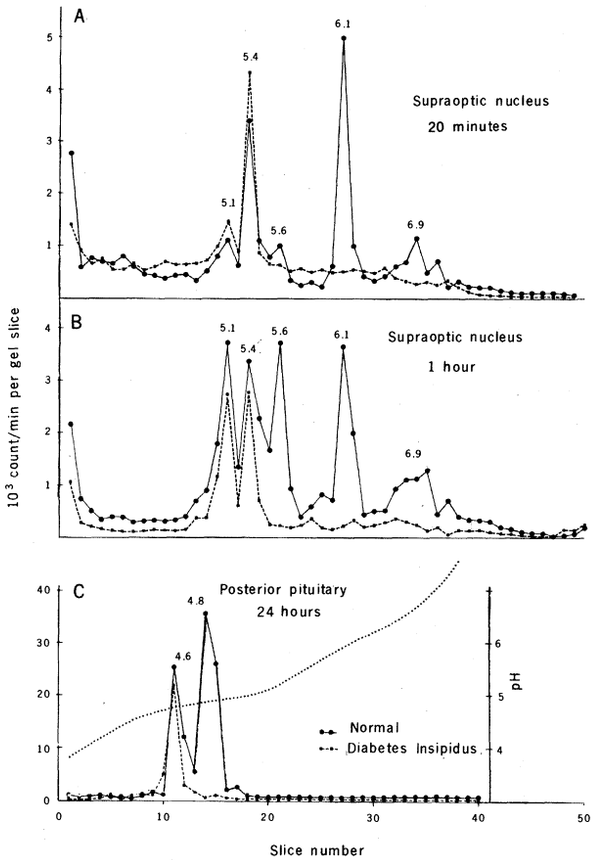

Fig. 4. Isoelectric focusing of ^{35}S-labeled proteins extracted from SON (A, B) and posterior pituitaries (C) of Osborne-Mendel rats given 2 percent saline to drink (solid lines) and Brattleboro rats (broken lines) after intracranial injections of [^{35}S]cysteine. Ampholytes from *p*H 3 to *p*H 10 were used for focusing the SON proteins; ampholytes from *p*H 4 to *p*H 6 were used to resolve the two pituitary peaks (neurophysins). The *p*I values of the protein peaks are indicated above them. The proteins from the Brattleboro rats lack the *p*I 4.8, 5.6, 6.1, and 6.9 peaks. (A) SON 20 minutes after injection. (B) SON 1 hour after injection. (C) Posterior pituitary 24 hours after injection. [From (*20*); courtesy of *Proceedings of the National Academy of Sciences of the United States of America*]

carboxypeptidase B (*25*), the tryptides would be longer than the nonapeptides. Furthermore, in the absence of an amidating enzyme, there was no reason to expect glycine amides to be formed. Thus, none of the above tests allowed us to distinguish between precursors that contain oxytocin and vasopressin. Consequently, it was essential that we study the Np-AVP (*p*I 6.1) and Np-OT (*p*I 5.4) precursors separately. The Np-AVP precursor could be separated from the Np-OT precursor chromatographically (*28*), and the Np-OT precursor could conveniently be labeled in Brattleboro rats.

When the purified Np-AVP precursor was incubated in trypsin (10 μg/ml) for 10 minutes at 37°C, it was almost completely converted to a 10,000-dalton material and peptides (Fig. 5, solid line) (*28*). A substantial fraction of the 10,000-dalton material did bind to vasopressin, and a substantial fraction of the peptide peak bound to neurophysin (29). The presumed neurophysin [LVP (lysine vasopressin)-bound 10,000-dalton material] was formed very rapidly, and more prolonged incubations with trypsin were deleterious to its binding to LVP-Sepharose. On the other hand, long incubations were required for the peptides to exhibit maximal binding to neurophysin-Sepharose.

Isoelectric focusing of the LVP-bound 10,000-dalton peak showed that it had the same *p*I as that of rat neurophysin (Fig. 6C); it had a *p*I of 4.8 in contrast to the precursor's *p*I of 6.1. Thus the 10,000-dalton peak generated from the purified *p*I 6.1 peak was identified as neurophysin, confirming our earlier suggestion that the *p*I 6.1 protein was a neurophysin precursor.

The neurophysin-bound peptides were chromatographed on Biogel P4 in the presence of 6*M* guanidine-hydrochloride (Fig. 6A). Since they eluted with vasopressin, the tryptic peptides had an appropriate size (about 1000 daltons). Furthermore, the peptides were bound by antibodies to vasopressin (Fig. 6B), and could be displaced by an excess of nonradioactive vasopressin. Therefore, the 20,000-dalton *p*I 6.1 neurophysin precursor contains a vasopressin-like peptide, which can be liberated by trypsin.

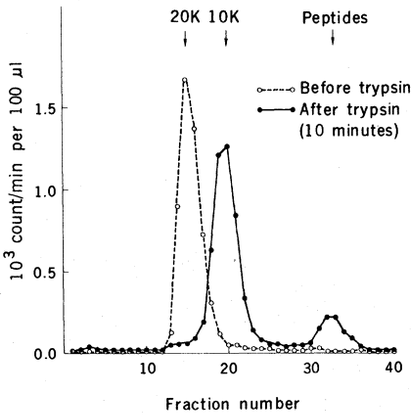

Fig. 5. Sephadex G-50 chromatography of the [^{35}S]cysteine-labeled 20,000-dalton putative Np-AVP presursor without trypsinization (the trypsin was added after acidification). The labeled 20,000-dalton peak is converted to a labeled 10,000-dalton peak and labeled peptides. [From (*28*); courtesy of *Proceedings of the National Academy of Sciences of the United States of America*]

As we pointed out earlier, we did not expect trypsin to cleave a molecule identical to vasopressin from the precursor. By using reverse phase high-performance liquid chromatography, we were able to show that the tryptic peptides that could bind to neurophysin and antibodies to vasopressin were heterogeneous (*28, 30*). Since a peptide must have a free amino terminus followed by tyrosine in the second position to bind to neurophysin, the vasopressin-like tryptic peptides must differ from vasopressin at the carboxyl terminus. We assume that the secretory granules contain all of the enzymes necessary for the complete processing of the precursor into vasopressin.

The *p*I 5.4 presumed Np-OT precursor found in the SON of normal and Brattleboro rats was studied in the same way that the *p*I 6.1 precursor was, with similar results. Trypsinization of the labeled *p*I 5.4 peak resulted in the formation of neurophysin-like and oxytocin-like molecules. Thus the *p*I 5.4 protein appears to be a common precursor for oxytocin and its related neurophysin. We have proposed that the vasopressin-Np-AVP precursor be called pro-pressophysin and that the oxytocin Np-OT precursor be called pro-oxyphysin (*31*).

Differences Between the Prohormones

When the labeled 20,000-dalton proteins in the SON of normal (but not Brattleboro) animals were passed over Con A (concanavalin A) on a Sepharose column support (a column that binds glycoproteins), most of the label was retained (*30*). This could be eluted with α-methylmannoside (Fig. 7). Isoelectric focusing of the bound material showed that it was the *p*I 6.1 precursor (pro-pressophysin). Our data are consistent with those of Lauber *et al.* who found that there was a high molecular weight molecule which reacted with antineurophysin antibodies and with Con A (*31*). Furthermore, we have shown that a fucosylated glycopeptide (7,000 to 10,000 daltons) is rapidly transported to the pituitary in

vivo along with neurophysin (which does not itself bind to Con A) (*32*). It seems probable that the rapidly transported glycopeptide is broken off of pro-pressophysin as it is processed, and we are purifying and characterizing this molecule further.

The *p*I 5.4 precursor (pro-oxyphysin) from normal and Brattleboro rats does not bind to Con A nor is there a fucosylated glycoprotein transported to the pituitary of Brattleboro rats. In contrast to pro-pressophysin, then, pro-oxyphysin is not a glycoprotein. The role of the carbohydrate on the pro-pressophysin and the significance of its absence from pro-oxyphysin are not clear.

Proposed Structure of Propressophysin

On the basis of the data that we have obtained up to now, we think that the structure of pro-pressophysin is most likely that in Fig. 8. We do not feel that the vasopressin can be on the amino terminus of the precursor because the precursor does not bind to neurophysin in a *p*H-dependent manner. The fact that relatively long incubation periods in the presence of trypsin are required to liberate AVP-like peptides with neurophysin binding sites also suggests that the vasopressin is extended in the precursor at the amino terminus. Since the peptides that finally do bind to neurophysin are heterogeneous, the vasopressin is extended at the carboxyl terminal as well. Thus, we have concluded that it resides in the middle of the precursor.

Neurophysin in contrast is liberated from pro-pressophysin rapidly. The neurophysin-like species that is generated by incubation with trypsin has a *p*I identical to that of the Np-AVP. Hence, it is unlikely to be extended at the carboxyl terminus, and this is consistent with its being on the carboxyl terminal end of the precursor. Therefore, the glycopeptide should form the amino terminus of the precursor. The validation of this proposed model of pro-pressophysin's structure must await determination of its amino acid sequence.

Number of Neurohypophysial Hormones and Where They Are Released

The anatomical boundaries of the hypothalamo-neurohypophysial system have expanded in recent years. Immunocytochemists have shown that the magnocellular neurons, especially those in the paraventricular nucleus, send fibers to the zona externa of the median eminence (*33, 34*), and to diverse areas of the central nervous system (*35, 36*). In addition, a third group of neurons that seem to contain vasopressin and neurophysin has been found in the dorsal pole of the suprachiasmatic nucleus (*34–37*); these parvicellular neurons project to the organum vasculosum laminae terminalis, lateral septum, periventricular nucleus, lateral habenula, and interpeduncular nucleus (*36*).

In order to find out what the various immunoreactive fibers secrete and whether they release vasopressin, oxytocin, and neurophysins, or whether they liberate other peptides, we looked at the

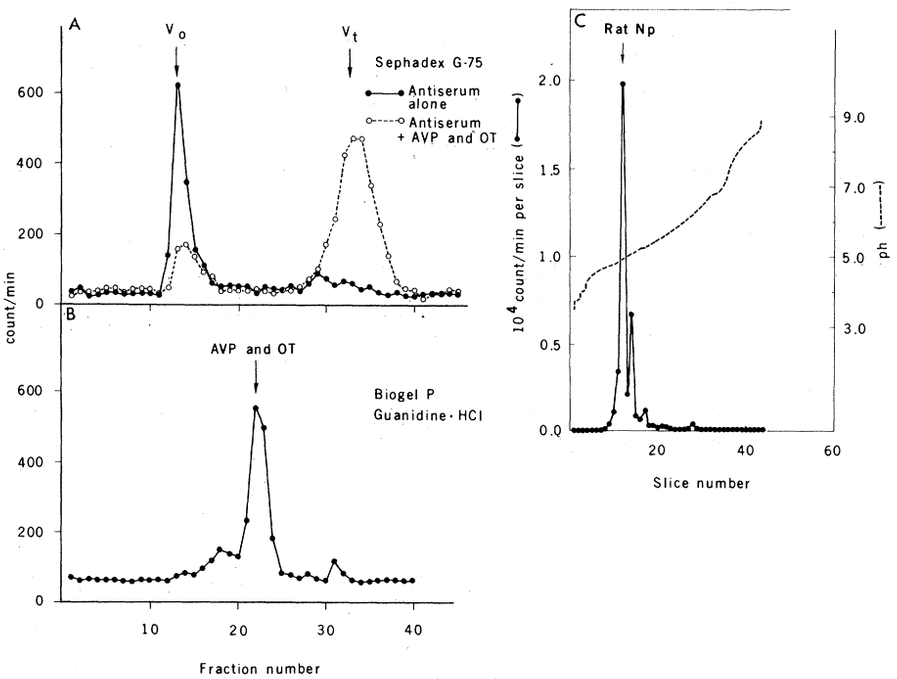

Fig. 6. Analysis of the peptide and 10,000-dalton tryptic products. The bulk of the labeled peptides liberated from the precursor by trypsin bound to neurophysin-Sepharose. These were eluted and incubated overnight with antiserum to LVP (lysine vasopressin) in the presence and absence of excess nonradioactive ligand. The mixtures were then chromatographed on G-75 Sephadex (A). V_o and V_t indicate the void volume and total volume of the column. In the absence of excess ligand, the peptides eluted in V_o bound to antibody. Molecular size determination on a Biogel-P4 column (B) showed that these peptides were about the same sizes as AVP and OT (arrow) (*28*). The 10,000-dalton peak that was generated by trypsinization of the 20,000-dalton precursor bound to an LVP-Sepharose column. The bound material had the same *p*I as neurophysin (C). [From (*28*); courtesy of *Proceedings of the National Academy of Sciences of the United States of America*]

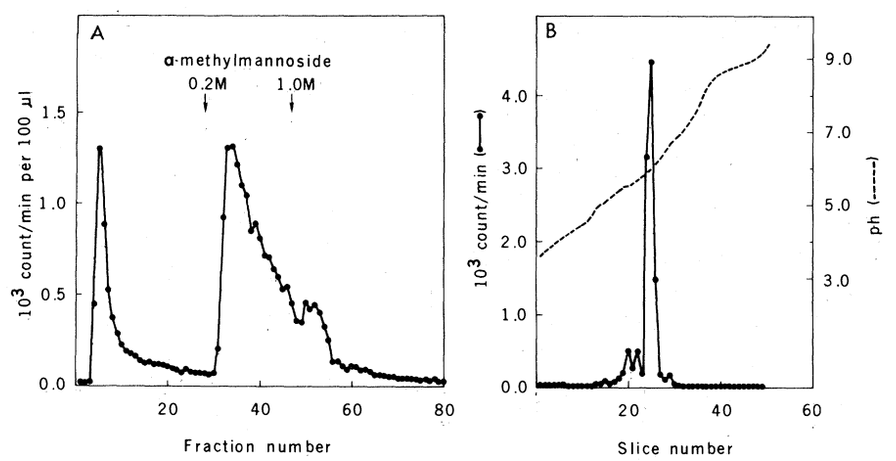

Fig. 7. Evidence that pro-pressophysin is a glycoprotein. (A) Affinity chromatography on Con A Sepharose of the labeled 20,000-dalton peak isolated from SON extracts by Sephadex G-75 chromatography. Most of the labeled protein isolated in this way binds to Con A Sepharose and is eluted by α-methylmannoside. (B) Isoelectric focusing of the Con A bound 20,000-dalton peak reveals that the label is primarily in the *p*I 6.1 precursor. No Con A bound labeled material is found in Brattleboro SON's (*38*).

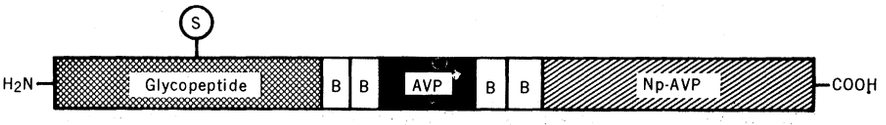

Fig. 8. Proposed structure of pro-pressophysin. Abbreviations: *S*, the sugar moiety of the glycopeptide; *B*, basic amino acid residues; *AVP*, arginine vasopressin; and *Np-AVP*, the vasopressin-associated neurophysin.

peptides that are transported to the pituitary and released from nerve endings there (*38*). [^{35}S]Cysteine was injected adjacent to the SON, and 1 day later labeled peptides were found in the posterior pituitary. Among these were ^{35}S-labeled peptides that bound to neurophysin-Sepharose and peptides that did not. Colchicine blocked the accumulation of the former, but not the latter. Thus, at least some of the unbound peptides appeared to be made in the posterior lobe itself, whereas all the bound peptides were transported there.

When posterior pituitaries were removed from rats 24 hours after intrahypothalamic injections of cysteine and placed in McIlwain-Rodnight buffer, there was a spontaneous leakage of labeled peptides from the pituitaries. Most of these peptides were not bound by neurophysin. Veratridine, an agent that depolarizes nerve endings, caused at least a tenfold release of peptides that bound to neurophysin, but it did not alter the rate of release of peptides that did not bind. The bound ^{35}S-labeled peptides released from normal and dehydrated rat pituitaries were identified as vasopressin and oxytocin by high-performance liquid chromatography. We have not observed other ^{35}S-labeled, bound peptides transported to and released by the posterior pituitary, but we have not yet studied Brattleboro rats (*39*). It will be important to do so because recently we have found that Brattleboro rats make a third neurophysin precursor (*38, 40*). This precursor comigrates with pro-pressophysin on Sephadex G-75, but it is not a glycoprotein. Trypsin cleaves this precursor into a neurophysin-like molecule and into peptides, some of which bind to neurophysin. Those tryptic peptides that bind to neurophysin migrate differently on reverse phase high-performance liquid chromatography from the peptide products of pro-pressophysin or pro-oxyphysin trypsinization.

The relation of the new putative neurophysin precursor to the other two precursors is unknown. It seems unlikely that it is an aberrant, nonglycosylated vasopressin precursor, however. It may be a vasotocin precursor, or it may give rise to an unknown biologically active peptide. The peptide products of the third precursor may be transported to the posterior pituitary to be released there, or may be released from terminals in the median eminence or extrahypothalamic sites. Only further biochemical studies will answer these questions.

Concluding Remarks

We have shown that peptide biosynthesis, transport, and release can be investigated in neurons that are anatomically well characterized using the experimental strategy that we have adopted for cell biological studies of the magnocellular neurons. Information about the neuroanatomical localization of a variety of biologically active peptides in combination with information about the biosynthesis, transport, and release of the peptides will help to elucidate the neurobiology of peptidergic pathways in the brain.

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40. We were able to detect this protein in Brattleboro rats because pro-pressophysin, which is chromatographically similar to it, is absent. Only one-fifth as much (counts per minute) of cysteine are incorporated into the new presursor as are incorporated into pro-oxyphysin.