NEUROHYPOPHYSIAL HORMONES

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I. INTRODUCTION

The description of the molecular structure of oxytocin in 1953 by du Vigneaud *et al.* (95) and Tuppy (340) initiated a period of intense activity in research on the pharmacology and physiology of neurohypophysial hormones. Many aspects of the field are discussed in detail in the contributions to the 1956 Colston Re-

search Society Symposium on the neurohypophysis (140) and to the 1959 Montevideo symposium on oxytocin (55). The present review will, therefore, emphasize more recent publications and particularly those dealing with active areas. Liberal use has been made of review articles and these should be consulted for additional references. Several recent reviews and monographs covering major portions of this subject are available (4, 21, 32, 46, 55, 78, 92, 124, 134, 140, 144, 173, 279, 293, 300, 309, 334, 347, 349).

II. SECRETION AND METABOLISM

A. Formation

That neurohypophysial hormones are actually formed within the hypothalamus and stored in the neural lobe is now generally accepted, principally on the basis of the work of the Scharrers (313) and Bargmann (18, 19, 20) and their colleagues. Large cells in the supraoptic and paraventricular nuclei, or in the preoptic nuclei of lower vertebrates, form neurosecretory material that stains characteristically with Gomori's chrome-alum-hematoxylin-phloxin and other stains. The neurosecretory products pass along the axons and are deposited in the nerve endings within the neurohypophysis. Normally these endings are dilated with stored neurosecretory material and are closely applied to the capillaries of the neurohypophysis.

Electron microscopy of the hypothalamo-neurohypophysial tract has revealed that the axons contain dense granules of 600 to 3,000 Å diameter (20, 22, 113, 127). These probably are the same neurosecretory granules revealed by Gomori's stain. Accumulation of granules in nerve endings, or proximally to levels of nerve transection, is taken as evidence that neurosecretory material is transported distally by axoplasmic flow (18, 19, 113, 313). Distal movement of granules within the living pituitary stalk has been observed directly by Carlisle (58).

Ultracentrifugation in sucrose solutions has been applied to neurohypophysial tissue (256). Oxytocic and vasopressor activities are found in small subcellular particles of size and appearance consistent with the dense granules seen on electron microscopy of the axons (145, 191, 199).

Neurosecretion in the hypothalamus and neurohypophysis is associated with the active neurohypophysial principles (18, 20). Appropriate physiological stimuli deplete concurrently stainable neurosecretion and assayable neurohypophysial activities (269, 304, 313). The active polypeptides that can be extracted from the neurohypophysis are probably not stained *in situ* by Gomori's method although they may take the stain under certain conditions (275, 313). It is more likely that the stain colors the protein to which the peptides are bound, the "carrier substance" (3, 18, 20, 155, 313). The stainable component can be dissociated from the active polypeptides by treatment of tissue sections with appropriate solvents. That only the carrier protein is stained is consistent with the presence of similarly staining neurosecretory material in many invertebrates in which neurohypophysial peptides are believed not to occur. The stain probably colors a carrier molecule associated with neurosecretory hormones that regulate many metabolic processes in invertebrates (311, 312, 313, 358).

The supraoptic nuclei of rats incorporate with extreme rapidity S^{35}-labelled cysteine injected into the subarachnoid space (320). This is interpreted as indicating uptake of the labelled amino acid into a protein or polypeptide actively synthesized in this region. After several hours labelled protein accumulates in the neurohypophysis where it is presumably stored in nerve terminals. The specificity of this uptake is emphasized by the observation that S^{35}-labelled methionine does not accumulate in the supraoptic nucleus or neurohypophysis. The neurohypophysial peptides and the protein with which they are associated are both rich in cystine but contain no methionine (43). Sulfur^{35}-labelled vasopressin can be isolated from the hypothalamus and neurohypophysis of dogs following the intravenous infusion of S^{35}-cystine (278). These observations confirm, in general, the hypothesis that neurosecretory products, including vasopressin, are formed by peptide synthesis in hypothalamic nuclei and that the products thus formed accumulate in the neurohypophysis.

B. *Chemical nature of the neurosecretory material*

The chemical form of neurohypophysial hormones in the hypothalamo-neurohypophysial system has been extensively investigated (4). Although the active principles are peptides with a molecular weight of about 1,000 they can be extracted from the neurohypophysis in combination with a protein of molecular weight about 30,000 (3, 4, 5, 8, 9, 10, 206, 351). Van Dyke *et al.* (351) believed that the protein they isolated contained both oxytocin and vasopressin, presumably one molecule of each in each molecule of protein. Constant solubility, homogeneity on electrophoresis and ultracentrifugation, and a fixed 1:1 ratio of oxytocic to vasopressor activity were taken as evidence that the protein was pure. More recently Acher *et al.* (8, 9) have isolated and studied the van Dyke protein. Vasopressin and oxytocin cannot be removed from the protein by dialysis against water but they are dislodged by dialysis against 0.1 N acetic acid, electrodialysis, trichloracetic acid precipitation of the protein, or countercurrent distribution. Since these processes do not hydrolyze peptide bonds it appears unlikely that the active peptides are fragments of the van Dyke protein. It is more likely that the protein represents the "carrier substance" to which the peptides are bound in a relatively loose association during transport and storage within the hypothalamo-neurohypophysial system.

The observation of van Dyke *et al.* (351) that the protein contained vasopressor and oxytocic activities in a 1:1 ratio reflects the source of their material. They worked with beef posterior lobes which contain, by definition, equal units of vasopressin and oxytocin. Such equality of vasopressor and oxytocic activities is by no means always found in extracts of the hypothalamus or neurohypophysis (142, 145, 350). This implies that the van Dyke protein may carry either oxytocin or vasopressin, or both. It is possible that there are separate secretory neurones for the synthesis of the two peptides (145).

Chauvet *et al.* (67) have investigated the inactive component of the van Dyke protein, which they call "neurophysin," from several species of mammals. They found that it can combine with oxytocin or vasopressin regardless of

whether the neurophysin or the peptides were derived from sheep, cows, horses, or pigs. The specificity of the association between neurophysin and the peptides is indicated by the observation that oxytocin, arginine vasotocin, and the vasopressins are the only peptides that accompany neurophysin in significant amounts in fractional precipitation of neurohypophysial extracts with NaCl (5, 8, 67, 68).

C. Release

1. Mechanisms. Neurohypophysial hormones can be rapidly released into the circulation in response to a wide variety of stimuli (32, 40, 73, 145). The cellular mechanisms of release are not clear. Stainable neurosecretion can be rapidly depleted (18, 20, 269). The dense granules seen by electron microscopy disappear. Empty vesicles are seen instead. The membranes that surround them may remain intact (113, 127, 358). Gross rupture of the encapsulated neurosecretions into the capillaries probably does not usually occur. The released products appear to pass through the membrane surrounding the granules and subsequently across both the cell wall and capillary endothelium (127, 145). This does not eliminate the possibility that the peptide-neurophysin complex is released intact, although it certainly suggests that the dissociation of the active peptides from neurophysin may precede their escape (358). Stimuli evoking hormone release, however, do cause disappearance of the stainable component of the neurosecretion. This suggests that neurophysin is also lost either by diffusion or by metabolism *in situ*. The rapidity of neurohypophysial responses does not rule out the diffusion of peptide combined with protein since the distances involved between neurosecretory endings and capillary are extremely small. The ease of rupture of the attachment between peptide and protein (8), however, suggests that such dissociation may free the active principles which can then diffuse from the nerve endings into the capillaries. The presence of droplets resembling neurosecretory granules has been observed in the capillaries of the neurohypophysis following stimulation (20). It is possible that neurosecretory material is released in droplets into the circulation under conditions that precipitate massive discharge of neurohypophysial hormones.

Nerve impulses passing down neurosecretory axons or other axons in the hypothalamo-neurohypophysial tract probably initiate physiological release of the hormones (73, 134). Vesicles, similar to those found in synapses, have been observed on electron micrographs of neurohypophysial nerve endings (113). It has been suggested that these vesicles release mediators which in turn precipitate release of the neurosecretory products. The absence of choline acetylase in the neurohypophysis, as demonstrated by Feldberg and Vogt (103), is strong evidence against the presence of acetylcholine in these fibers and its participation as a mediator of the release of hormone from neurosecretory nerve endings.

The current view holds that the pituicyte is simply a supporting structure and does not participate in the manufacture of the neurohypophysial hormones (18, 20, 269, 313). Leveque and Small (202) have recently observed increased mitotic activity in the pituicytes of the rat after dehydration or hypertonic saline administration. They suggested, as did Gersch (112) and others, that the

pituicytes may participate, in some manner, more actively than as mere architectural support. More evidence is necessary before this concept can be confirmed or denied.

2. Selective release of individual hormones. Effective stimuli, in most instances, appear to provoke the release of both oxytocin and vasopressin into the circulation (40, 73, 134, 145, 261). How much independent control exists over the secretion of either hormone is still subject to debate. There are relatively few investigations on this subject that involve valid biological assays for both hormones released into the circulation. Hemorrhage in rats causes a marked elevation of the vasopressin concentration in internal jugular vein blood (122) without a comparable increase in oxytocin. Hemorrhage in rabbits does not cause detectable increase in oxytocin in the peripheral blood (65), although it is known to be a potent stimulus for vasopressin release in other species (17, 119, 120, 357). Anesthetic drugs and nicotine appear to release much more oxytocin than vasopressin into the jugular vein blood of rats (40) and the peripheral blood of rabbits (65). Nicotine in man also releases both oxytocin and vasopressin although not in what appears to be a fixed ratio (40).

Experiments utilizing responses of animals to endogenously released hormones indicate that electrical stimulation of the hypothalamus and osmotic stimuli cause the release of oxytocin in excess of vasopressin (2, 13, 73, 134). The ratios of oxytocin to vasopressin estimated vary quite widely, from 4:1 to about 100:1 (134). These estimates are based, however, on responses to various stimuli in different species.

Suckling has been reported to cause antidiuresis in lactating mammals. This suggests simultaneous release of vasopressin and oxytocin (134, 258, 262). "Suckling antidiuresis" is not always associated with let-down in cows (258) and is usually absent in women (331). The milk-ejection reflex does not therefore appear invariably to evoke significant release of vasopressin coincident with the release of oxytocin.

Many investigators have measured the hormonal activities remaining in the neurohypophysis after stimulation (142, 349, 350). On the basis of such experiments it appears that dehydration or the infusion of hypertonic saline in rats can deplete both oxytocin and vasopressin (145, 269, 304). Lactation in rats, on the other hand, has been reported to reduce oxytocin more than vasopressin (9). Vasopressin can apparently also be reduced in lactating rats (269) or dogs (349). Data of this type must be interpreted with caution since the assayable activities remaining in the hypothalamo-neurohypophysial tract represent a balance between the rates of release and repletion. These rates for each hormone may vary quite independently.

Estimates of the release of oxytocin and vasopressin in different circumstances vary considerably and often appear contradictory. It seems probable that there is a certain degree of independent control over the release of the two major neurohypophysial principles (261). Release of either in the complete absence of release of the other cannot be satisfactorily established since the methods available are, in general, too insensitive (40, 73, 145).

D. Factors influencing release

Direct stimulation of the neurohypophysis, the tracts leading into it, and many other areas of the brain can precipitate release of neurohypophysial hormones (13, 73, 134, 261). Many drugs are also capable of causing hormone release. Notable are nicotine, acetylcholine, hexamethonium (41), lobeline (128), morphine, several tranquilizers (42), and many anesthetic agents (40, 65, 150). The action of nicotine is not blocked by doses of hexamethonium that effectively block other nicotinic responses (41). Ethanol can inhibit vasopressin secretion in response to osmotic stimuli and can, therefore, produce inappropriate water diuresis (186). It does not, however, inhibit the response to nicotine in rats (40).

Responses of the neurohypophysis to osmotic stimuli are of more immediate physiological interest. Hypertonic saline injected into the carotid circulation can produce antidiuresis (172, 354, 369), milk ejection (158), and uterine contractions (2, 319). Hypertonicity of plasma is believed to stimulate osmoreceptors somewhere in the thalamus or hypothalamus (172). The exact nature and location of such osmoreceptive elements remain unknown. It is possible that the neurosecretory neurons themselves can respond to alterations in the effective osmotic pressure of the surrounding fluids. Regardless of which cells actually detect concentration changes, those responsible for regulating the release of neurohypophysial hormones appear to be within the diencephalon (172). Hypertonic saline injected into the carotid artery of rabbits with "diencephalic islands" provokes the same degree of milk ejection as in rabbits in which the hypothalamus is connected to other parts of the brain (329). Hypertonic saline as well as nicotine can inhibit water diuresis in cats with similarly isolated hypothalami (366).

Direct microinjection of solutions of varying osmolarity into the hypothalamus also influences milk ejection and antidiuresis in a manner indicating that vasopressin and oxytocin release are influenced by osmoreceptors in or adjacent to the hypothalamic nuclei in which the hormones are formed (13). Such application of hypertonic saline cannot, however, be considered a necessarily specific stimulus.

Action potentials can be evoked in many parts of the brain by the intravascular injection of hypertonic solutions (70, 73, 159, 328). It would seem unlikely that all the reactive areas contain osmoreceptors concerned with the release of neurohypophysial hormones. Intracarotid infusions of hypertonic saline in rabbits do, however, evoke electrical activity in neurons of the supraoptic nuclei that is temporally related to milk ejection (74). This is probably more than a coincidence and these potentials may reflect excitation of neurons, either directly or indirectly, associated with the release of oxytocin.

Infusion of hypotonic saline through the cerebral ventricles of dogs that have not been water-loaded can initiate water diuresis (201). There is a lag of about 30 minutes from the onset of infusion. A similar latent period is observed when water is administered to a nondiuretic animal. The delay is probably necessary for the metabolism of previously secreted vasopressin and for renal recovery

from its action. These experiments are consistent with the view that osmoreceptors can respond to dilution of their environment by inhibiting vasopressin release.

Expansion of the circulating blood volume can also induce water diuresis (322, 359). Manipulations that increase intrathoracic blood volume appear particularly effective. Negative pressure breathing induces profuse water diuresis in dogs (111) and men (51, 167). This diuresis is presumed to be due to suppression of vasopressin release, since glomerular filtration rate and solute excretion are not significantly increased. Evidence has been reported indicating that stretch receptors in the left atrium of dogs can, when stimulated, inhibit vasopressin release (154). Afferent inhibitory impulses appear to travel in the vagi. Stimulation of the central end of the cut vagus was reported 20 years ago to result in apparent release of vasopressin and oxytocin (63, 64). Afferent impulses from the viscera may, therefore, either inhibit or stimulate release of neurohypophysial hormones. Positive pressure breathing has been reported to increase the level of vasopressin assayed in the peripheral blood of dogs (17).

It appears likely that there are many receptors in blood vessels and viscera that influence the hypothalamic regulation of hormone release (322, 359). Some may be directly concerned with the maintenance of body fluid volume and circulatory homeostasis. Other afferent impulses, such as pain, also influence neurohypophysial activity. The physiological importance of neurohypophysial hormone release in response to pain is not understood (73, 86, 145).

Abrupt decreases in circulating blood volume, as in hemorrhage or cuffing of the extremities, stimulate the release of neurohypophysial hormones. Massive hemorrhage is the most powerful known stimulus, and extraordinarily high concentrations of vasopressin can be found in the blood of rapidly exsanguinated animals (17, 85, 119, 193, 357). It is possible that vasopressin is released under these circumstances in quantities sufficient to effect vasoconstriction, particularly of the capillaries, and thereby has some adaptive value in decreasing further blood loss.

Dehydration, acting perhaps by both increasing plasma osmolarity and decreasing blood volume, is an important natural stimulus for vasopressin release. If continued there is depletion of stainable neurosecretory material (18, 20, 166, 269, 313) as well as a decrease in vasopressin and oxytocin in the neurohypophysis (145, 269, 349). Increased antidiuretic activity of plasma from dehydrated men and rats has been reported by J. Heller and Štulc (152, 153). The amounts estimated are so small that they would escape detection by most assay techniques. However, these authors stated that their modification of the intravenous rat antidiuretic assay (151) is at least ten times as sensitive as any previously published technique. It is difficult to understand, however, how they could discern such minimal responses and discriminate between hormone concentrations in the range in which they worked. They have not applied chemical or chromatographic criteria to plasma samples to support the contention that they are actually assaying plasma vasopressin. Plasma from hypophysectomized animals has been reported to show appreciably more antidiuretic activity, when assayed by a

similar technique (85), than the normal "antidiuretic hormone" concentrations found by Heller and Štulc (152, 153). Baratz *et al.* (16) could not detect antidiuretic activity by a sensitive intravenous rat assay in superior vena caval blood from dehydrated normal men. Even nicotine or hypertonic mannitol administered to normal subjects did not always raise the antidiuretic titer of caval blood into the range of their assay (over 30 μU/ml) even when sufficient hormone was released to cause sharp antidiuresis. Schröder and Rott (315), using a similar assay, found that peripheral blood from dehydrated subjects contained less than 20 μU/ml. The problems involved in attempting vasopressin assay in plasma are formidable. These have been reviewed elsewhere (291, 349). Reliable determinations of plasma vasopressin concentration in the range encountered in moderate dehydration are probably possible only after considerable concentration and partial isolation of the active hormone (16, 291).

Fitzpatrick (105) has succeeded in measuring oxytocin in the jugular blood of ewes during delivery. He found very high levels of hormone during the second stage. There is rapid diminution in secretion after delivery of the placenta. Oxytocin release during delivery may result in part from reflexes arising in the cervix and adnexa. The role of oxytocin release in the initiation of labor has not been established (71, 72). Certainly cervical or vaginal stimulation can cause oxytocin release under appropriate conditions (32, 73, 80, 104).

Oxytocin is secreted in response to suckling (73, 105, 258). This is essential for making milk available to the infant. Tactile stimulation of the nipple is usually considered responsible for the milk-ejection reflex although other influences are certainly involved (73, 258). Sheep and goats have been reported capable of nursing their young adequately after apparently complete denervation of the nipples (83). This may indicate that tactile stimulation of the teats is not absolutely essential for the release of oxytocin and milk ejection in these species.

E. Hormones in blood and urine

Although neurohypophysial hormones may be released as free peptides they may not circulate as such (141, 334). Plasma antidiuretic activity of endogenous origin in rats and dogs appears to be bound in part to a large molecule. Most of the activity in rat plasma is not ultrafilterable (338). In the dog, however, approximately 70% appears to be free (193). Antidiuretic activity in rat serum was found to move with the beta globulins in zone electrophoresis by Thorn and Silver (338). Arginine vasopressin and oxytocin can be bound to rat serum globulins *in vitro* (335). The active peptides can be dissociated from binding by prolonged dialysis against water. This is not true of the peptide-neurophysin complex. Serum antidiuretic activity is rapidly dialyzable against 0.2 N acetic acid.

Thorn and Silver's (338) observations suggest that neurohypophysial peptides are partially bound to a large plasma protein in an easily dissociable form. Injected hormones often appear distributed in a limited volume. This could be due to such protein binding (141, 334). Some authors, however, have observed that injected peptides diffuse into volumes approaching that of the total body water (88, 105, 122), indicating that such binding is neither complete nor irreversible.

There are reports that endogenous antidiuretic hormone appears in the urine in a nondialyzable form (141, 336, 349). It is questionable whether this represents the excretion of the intact serum protein-hormone complex. It is just as probable that free peptides are excreted and subsequently adsorbed onto urinary protein (336).

F. Inactivation and excretion

1. Clearance studies. The important studies of Ginsburg and Heller (121) indicate that exogenous vasopressin injected into rats is inactivated principally in the liver and kidneys. If these are excluded from the circulation, the rate of disappearance of vasopressin is markedly decreased (121, 141, 143). Dicker and Nunn (88) could not confirm this in rats, but Lauson and Bocanegra (193) have demonstrated the importance of hepatic and renal inactivation of vasopressin in dogs. Similar evidence has been obtained concerning inactivation of exogenous oxytocin in rats (122) and rabbits (66). Such experiments must be viewed with caution, however, since large doses of neurohypophysial principles are administered in order to achieve plasma levels adequate for biological assay. Lauson and Bocanegra (193) have tried to avoid this by using plasma containing endogenously liberated antidiuretic hormone. Such plasma exerts less activity in the recipient dog if injected into the hepatic or renal circulation than if injected intravenously. Ginsburg (118) also found that the vasopressor response to vasopressin infused into the portal vein of rats is much less than after intravenous injection elsewhere. Such comparisons indicate that both liver and kidneys can be significant sites of removal of hormone from the circulation.

The estimated renal clearance of exogenous vasopressin by the dog kidney is approximately 40 to 65% of the total plasma flow (193). Hepatic clearance is about 5 to 15% of hepatic portal flow. In rats, urinary vasopressin clearance exceeds glomerular filtration rate (118). Less than a quarter of the total vasopressin cleared appears in the urine; the remainder is probably inactivated.

As much as 25% of the vasopressor activity of infused Pitressin may appear in the urine of normal rats (88). Eviscerated rats, however, excrete only about 5% of the infused activity, according to Dicker and Nunn (88). This might be due to failure of renal function in eviscerated rats. These authors, however, suggested that the intact rat alters vasopressin so that a larger portion escapes renal inactivation and appears in the urine. In support of this hypothesis they reported that if urine from intact rats containing vasopressor activity is subsequently infused into another intact rat the second rat excretes about 60% of the infused activity. Heller (141) could not confirm this. It is unfortunate that these conflicting experiments were performed using Pitressin, which is made from pooled beef and hog pituitaries and, therefore, contains an uncertain mixture of arginine and lysine vasopressins. The renal responses of the rat to these vasopressins are clearly different (244, 288) and the degree of renal destruction may also differ. These experiments deserve repetition with hormones of known origin.

Exogenous neurohypophysial hormones disappear from the circulation extremely rapidly (32, 141). Half-lives for circulating vasopressin have been estimated at about 1 minute in rats (118, 324), 3 minutes in rabbits (66), and about

8 minutes in man (315). Half-lives for exogenous oxytocin have been estimated at 1.65 minutes in rats (122), 3 minutes in rabbits (66) and pregnant women (123), and less than 1 minute in sheep and man (105). These estimates are based on assay of circulating hormones following injection. Estimates based on the rate of decay of responses to injected hormones do not necessarily reflect the rate of hormone metabolism. They depend also on the rate at which the reacting tissue can regain resting levels. Such estimates may be, therefore, considerably longer than those based on assays of circulating hormones (69, 318).

2. Inactivation in vitro. Neurohypophysial hormones are removed from the circulation by rapid inactivation in the kidneys and liver. Both peptides are irreversibly destroyed by incubation with homogenates of liver or kidney (149). "Tubular" tissue from the kidney appears more active than "glomerular" tissue in destroying oxytocin and vasopressin (66, 149). Destruction appears to be enzymatic, as it can occur in cell-free extracts and is prevented by previous boiling of the extracts. Inactivation of oxytocin can also occur at much slower rates in homogenates of rat skeletal muscle and nonpregnant uterus (284). Audrian and Clauser (15) have recently reported that nonpregnant rat uterus homogenates do not inactivate oxytocin under aerobic conditions. Anaerobic destruction may be a dual process. The initial step appears to be reversible reduction of the disulfide bridge. The second step is irreversible inactivation of previously reduced oxytocin. The second step can be brought about by an enzyme released into the incubation medium. Skeletal muscle exhibits similar behavior. Liver or kidney homogenates, however, inactivate oxytocin aerobically as well as anaerobically (15). This suggests that the enzymatic systems involved in liver and kidney differ from those in nonpregnant uterus or skeletal muscle.

Considerable work has been done on the destruction of oxytocin by tissues from lactating and pregnant animals (32). Ginsburg and Smith (122) noted that there is significant destruction of oxytocin by tissues other than kidney and liver in lactating rats. They suggest that this occurs in the mammary glands. Sawyer (284) reported that homogenates of myometrium from pregnant or pseudopregnant rats destroy oxytocin at rates comparable to the rates of inactivation by liver or kidney. Skeletal muscle, plasma, and nonpregnant myometrium show little activity.

Oxytocinase activity in the rabbit uterus, however, decreases during pregnancy and is minimal at term (90). This oxytocinase, and that from pregnant rat uteri, are nondialyzable, heat-labile, and can be precipitated with ammonium sulfate. Oxytocinase activity was not found in myometrium from several women near term (90).

Oxytocinase and vasopressinase from human placenta have been studied by Hooper (163, 164). Both appear to be peptidases. They have different pH optima and can be separated by fractional centrifugation in sucrose solutions (165). Oxytocinase remains in the supernatant while vasopressinase activity is found primarily in the fractions containing mitochondria and microsomes. Placental vasopressinase can be partially separated from the particulate matter by lysis (164). Pretreatment with an anionic detergent followed by tryptic digestion

renders the enzyme soluble. This may indicate that it is associated with a lipoprotein in the cell particles. Di*iso*propyl phosphofluoridate (DFP) and tetraethyl pyrophosphate (TEPP) inhibit oxytocinase but not vasopressinase (163). Aeration, CO, N_2, CN^-, iodoacetate, *p*-chloromercuribenzoate and ethylenediamine tetraacetic acid (EDTA) do not inhibit either enzyme, suggesting that they do not resemble dehydrogenases or oxidases, and that they do not depend on the presence of metallic ions or free SH–groups (163).

High levels of circulating oxytocinase and vasopressinase are found in human plasma during pregnancy (32, 234) but not in plasma from nonpregnant women or men. Plasma oxytocinase has not been found in blood of nonprimate mammals, even during pregnancy. It has been reported that human pregnancy plasma can bind vasopressin in an inactive form that can be freed by boiling (157). This is not in accord with Tuppy's (341, 342) assertion that the vasopressinase in pregnancy plasma is identical with oxytocinase. His studies show that plasma oxytocinase is an aminopeptidase that opens the oxytocin ring by cleaving the bond between the N-terminal half-cystine and tyrosine. This bond is also found in vasopressin. Opening of the ring of either vasopressin or oxytocin causes irreversible destruction of their pharmacological activities.

Tuppy (341, 342) has also studied the ability of plasma oxytocinase to hydrolyze L-cysteine-β-naphthylamide, releasing free β-naphthylamide which can be measured colorimetrically. Unlike tissue oxytocinases, plasma oxytocinase is inhibited by EDTA. A variety of peptides containing S-benzylcysteine can also inhibit plasma oxytocinase (30, 31). These peptides are themselves split if they contain a cysteine-tyrosine bond. Peptidases from pancreas and liver that destroy oxytocin are inhibited by a wider variety of peptides and by free cystine. Many inhibitory peptides that do not resemble amino acid sequences found in oxytocin may be hydrolyzed by these tissue peptidases. The structural specificities of these tissue enzymes are, therefore, not clear.

In summary, enzymes capable of destroying oxytocin or vasopressin or both exist in a great many tissues (341). Their properties differ considerably depending on the tissue, the species and the reproductive state of the animal. The specificity of these enzymes to the neurohypophysial peptides is not incontrovertibly established in any case. Most studies suggest that they are aminopeptidases. Enzymes in the kidney and liver are undoubtedly important in the metabolism of endogenous neurohypophysial hormones. The contribution of oxytocinases found in the blood, placenta, or myometrium of pregnant animals to the protection of the uterus from premature excitation has certainly not been determined.

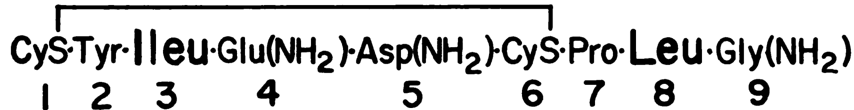
III. ACTIVE NEUROHYPOPHYSIAL PEPTIDES

A. *Natural principles*

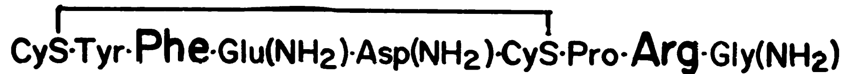
1. Oxytocin. The determination of the molecular structure of oxytocin (Fig. 1) in 1953 (95, 340) opened an era of rapid progress in neurohypophysial physiology. Oxytocin was soon synthesized by du Vigneaud *et al.* (94). The vasopressins and many structural analogues of the natural hormones were synthesized subsequently.

Oxytocin has been isolated and its identity confirmed by amino acid analysis

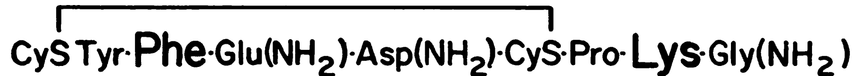
Oxytocin



Arginine vasopressin



Lysine vasopressin



Arginine vasotocin

FIG. 1. The molecular structures of the four known natural neurohypophysial hormones
The amino acids are numbered as if these were straight-chain nonapeptides, in order, starting with the N-terminal half-cystine. This numbering system is used in the text and the tables as a means of identifying positions on the molecules.

in neurohypophysial extracts from cattle, pigs (264), men (205), horses (5), sheep (67), and chickens (6). Pharmacological studies indicate that it is present in all the bony vertebrates that have been studied with one exception (298): a pituitary extract from young caimans (*Caiman crocodilus*) contained little apparent oxytocin (303). Oxytocin may be present in extracts of pituitaries of young mammals in relatively small amounts (89, 146). Since other reptiles, the green turtle and the iguana, have oxytocin, as do chickens, amphibians and teleost fishes (293, 294, 299, 302, 303), the lack in young caimans would not appear to be of evolutionary importance.

Oxytocin does not appear to be present in a cyclostome (*Petromyzon marinus*) (299, 302). Since this is a very primitive vertebrate the absence of oxytocin in adults suggests that oxytocin may have appeared later in vertebrate phylogeny.

Extracts of neurointermediate lobes of elasmobranchs contain an oxytocin-like principle (259, 299). The pharmacological properties of such extracts could not be due to oxytocin alone (302) but suggest the presence of a peptide closely related to oxytocin.

2. The vasopressins. Arginine vasopressin was originally isolated from ox pituitary extracts and its structure determined (Fig. 1) (91). It was subsequently synthesized. It has also been isolated and its constituent amino acids have been

identified in neurohypophysial extracts from man (205), horse (5), and sheep (67). Chauvet *et al.* (68) have also isolated a peptide containing the amino acids of arginine vasopressin from chicken neurohypophysial extracts to which horse neurophysin had been added to facilitate precipitation. The pharmacological and chromatographic studies of Munsick *et al.* (242) and Pickering and Heller (260), however, indicate that very little arginine vasopressin, if any, can be present in crude chicken extracts. Further investigation is necessary before the existence of arginine vasopressin as a natural hormone in the chicken can be considered established.

Arginine vasopressin has been identified by pharmacological methods in neurohypophysial extracts from monkeys, cats, dogs, rabbits, several rodents, camels (350), peccaries (293), opossums, and spiny anteaters (301).

Lysine vasopressin (Fig. 1), rather than arginine vasopressin, is the vasopressor principle in neurohypophysial extracts from the domestic pig (91). It has also been identified pharmacologically in an extract of hippopotamus neurohypophysis (147).

3. Arginine vasotocin. Arginine vasotocin is an analogue of mammalian neurohypophysial hormones. It has the ring structure of oxytocin and the side-chain of arginine vasopressin (Fig. 1). It was synthesized by Katsoyannis and du Vigneaud (180) in the expectation that its pharmacological properties would supply valuable information on the relationships between the molecular structures of the natural peptides and their biological activities. It has relatively strong vasopressor and oxytocic activities, approximately ¼ and ⅒ the respective activities of arginine vasopressin and oxytocin (180).

Arginine vasotocin was found to have extraordinary potencies when assayed for its ability to cause contraction of the fowl oviduct (242, 298, 353), to increase the water permeability of the frog bladder (171, 290, 298), to inhibit diuresis in the frog (171, 344) and hen (242, 298, 353), and to promote sodium transport by the isolated frog skin (171, 292). Comparison of these activities of synthetic arginine vasotocin to the respective activities in neurohypophysial extracts led to the conclusion that this analogue is actually a natural neurohypophysial principle in most nonmammalian vertebrates (171, 293, 298, 300). Its pharmacological properties suggest that it is identical with Heller's (139) "amphibian water-balance principle" and with the "natriferin" (171, 292) of Maetz *et al.* (218).

A peptide containing the amino acids of arginine vasotocin has been isolated from neurohypophysial extracts of chickens (68), frogs (7), and pollack (148). The presence of arginine vasotocin as a natural peptide in nonmammalian vertebrates appears to be well established (144). Katsoyannis and du Vigneaud (180) had, therefore, synthesized a polypeptide hormone before its existence in nature had been recognized (182).

B. Evolution

The most primitive living vertebrates, the cyclostomes, appear to have arginine vasotocin (293, 298, 299, 300, 302). Since neurohypophysial peptides are not

known to occur in invertebrates (289) we can assert that this is the most primitive known peptide. The elasmobranchs appear to have evolved a different active peptide (259, 299, 302). It has strong pharmacological resemblance to arginine vasotocin. Consideration of this resemblance suggests that it may differ from arginine vasotocin only in the substitution of an amino acid other than arginine, lysine, or leucine in the 8-position (299, 302).

A principle which resembles oxytocin appears in the bony fish (260, 298, 299, 302). Oxytocin could arise simply by the substitution of 8-leucine for the 8-arginine of arginine vasotocin. Oxytocin appears to exist in neurohypophyses from representatives of all bony vertebrate classes (144, 293).

The function of arginine vasotocin in fishes is not known. It appears to be the antidiuretic hormone of nonmammalian tetrapods (300). Monotremes, marsupials, and most placental mammals have arginine vasopressin as their antidiuretic hormone (293, 301). This implies that arginine vasopressin appeared very early in mammalian evolution. Since monotremes are believed to have evolved from different therapsid ancestors than did marsupials and placental mammals, it is probable that arginine vasopressin first appeared in premammalian reptiles (293, 300, 301). The modern reptiles that have been examined, however, possess arginine vasotocin. Unfortunately, no reptiles closely related to the ancestors of the mammals survive. No further progress in locating the phyletic juncture at which arginine vasopressin displaced arginine vasotocin would appear possible.

The pig and the hippopotamus both possess lysine vasopressin rather than arginine vasopressin (144, 147). It is surprising, therefore, that the peccary, thought to be more closely related to the Old World pigs than is the hippopotamus, appears to have arginine vasopressin (293). It is possible that this represents a reverse mutation in that lysine vasopressin may have displaced arginine vasopressin early in the phylogeny of the pig group, only to have arginine vasopressin reappear in the ancestors of the living peccaries. It is also possible that the peccaries may not be as closely related to the Old World pigs as has been commonly believed; they may have separated from the ancestral line leading to the pig and hippopotamus very early in the evolution of the artiodactyls.

IV. RELATIONS BETWEEN STRUCTURE OF PEPTIDES AND THEIR PHARMACOLOGICAL ACTIVITIES

A. General

A remarkable feature of the known natural neurohypophysial peptides is that they share 6 amino acids in common and that these occupy the same relative positions (Fig. 1). The natural peptides differ only in the amino acids in the third and eighth positions in the chain.

In this, and subsequent discussions, the numbering system introduced by Konzett and Berde (189) and Bodanszky and du Vigneaud (44) will be used to designate amino acid positions (Fig. 1). The number will precede the name of the amino acid (44). Konzett and Berde (189) placed the number as a superscript following the adjectival form of the name of the amino acid. This latter system does not allow for distinction between a substitution in the chain and an amino acid attached as a side-group to the chain. Bodanszky and du Vigneaud

(44) used the adjective to designate such a side-group. In their system 1-glycyl oxytocin indicates that a glycyl residue has been attached to the half-cystine in the 1-position of oxytocin. In Konzett and Berde's (189) terminology glycyl^1-oxytocin would be oxytocin in which glycine has been substituted for the half-cystine in the 1-position, and would be synonymous with 1-glycine oxytocin in the Bodanszky and du Vigneaud (44) nomenclature.

Questions have arisen concerning the nomenclature that should be applied to the natural mammalian neurohypophysial peptides as well as to their synthetic analogues. Some feel that the word "vasopressin" is a poor choice since it derives from a pharmacological property of uncertain physiological significance. The name is, however, historically established since the vasopressor response to posterior pituitary extracts was the first to be described. The molecular structures of the active vasopressor principles were also first described under the classical name (91). Obviously, one must add a preceding "arginine" or "lysine" in order to establish the chemical identity of the vasopressin concerned. But this would also be true if one substituted "antidiuretic hormone," "ADH," or "Adiuretin." The use of such names based on the antidiuretic response is also complicated by the fact that there is at least one antidiuretic hormone that is not a vasopressin (300). It would seem wise, therefore, to keep the established name "vasopressin" on the conservative bases that it is familiar, has some chemical meaning when properly modified by "arginine" or "lysine," and no obviously preferable name has been proposed.

When designating substituted analogues it is usually convenient to consider them as derivatives of oxytocin (189). Since both arginine and lysine vasopressins are well-defined entities, however, it is often simpler to consider some analogues as derivatives of vasopressin. Katsoyannis and du Vigneaud (181), for example, named an analogue "histidine vasopressin." This could be called, more exactly, "8-histidine vasopressin." This is easier than the alternative "3-phenylalanine, 8-histidine oxytocin." It appears justified to allow the name based on vasopressin to stand in the interest of clarity and economy since identity has not been sacrificed. Although other analogues have been labelled with hybrid names such as "oxypressin" (179) or "arginine vasotocin" (180), it is probably prudent not to use these names as bases for the nomenclature of further substituted analogues since it is unnecessary and could lead to further confusion.

Du Vigneaud and his co-workers at Cornell (44, 93, 96, 179, 180, 181, 194, 209, 226, 227, 270, 271) and Boissonnas and his colleagues at Sandoz, Basle (33, 34, 35, 36, 45, 47, 49, 50, 133, 170, 188, 189), have prepared and studied many synthetic analogues of the natural neurohypophysial hormones. The possible combinations of amino acids in a nonapeptide chain are almost infinite. The natural peptides, however, differ only in the constituents present in the 3- and 8-positions (Fig. 1). The Sandoz group in particular (34, 45, 46, 188) has focussed its attention on the synthesis of peptides that differ from the natural hormones in the constituents in the 2- and 3-positions. The pharmacological properties of such analogues afford considerable information on the structural specificities of several biological responses. This information is summarized in the tables and discussed briefly in subsequent sections.

Professor du Vigneaud and his colleagues have not only synthesized several such substituted analogues but they have also devoted great attention to the effects of other alterations in the basic molecular structures of the peptides. Particularly interesting are the results of manipulation of the ring structure and of covering or removing reactive groups on the constituent amino acids.

Reduction of the S–S bond of the 1-6-cystine results in loss of all activities of neurohypophysial peptides (270). Such reduction is reversible after treatment with cysteine (317) but not after thioglycollate (40, 351). The ring structure containing the intact disulfide bond appears essential to all activities. The ring of oxytocin without the terminal tripeptide (Pro-Leu-Gly(NH_2)) chain retains some oxytocic and milk ejection activities (270) but is about $\frac{1}{100}$ as strong as oxytocin itself (Tables 4, 5). Dimers formed by condensing portions of two oxytocin chains by S–S cross-linkage between the 1- and 6-cysteine residues are inactive (34, 270).

The exact size of the oxytocin ring, containing 20 atoms, appears to be critical to biological activity. Enlarging the ring by adding one or two methyl groups, by substituting 4-*iso*glutamine for the 4-glutamine, or 5-*iso*asparagine for the 5-asparagine (209, 271), eliminates oxytocic and other activities. Duplicating an amino acid, and correspondingly increasing the size of the ring by 3 atoms, by inserting two tyrosines in place of the 2-tyrosine, or two isoleucines in place of the 3-isoleucine, also eliminates pharmacological activity. Reduction of the ring by one amino acid, by omitting isoleucine and thereby reducing the size to 17 members, also destroys the activity of oxytocin (45). Reduction by only one atom cannot be easily accomplished in oxytocin itself. Du Vigneaud *et al.* (96) have observed that reduction of the ring of desamino oxytocin by one member decreases its fowl vasodepressor activity by 99.9%.

Many substitutions within the ring that do not change the length of the cyclic peptide also appear incompatible with the pharmacological activities of neurohypophysial peptides. Substitution of 5-glutamine for 5-asparagine, or of 2-serine or 2-histidine for 2-tyrosine, essentially eliminates vasopressor or oxytocic activities (34). The only substitution at the 2-position that appears compatible with appreciable pharmacological activity is that of 2-phenylalanine for 2-tyrosine (44, 47). This represents merely the removal of the *p*-hydroxyl group of tyrosine. It does significantly reduce oxytocic, milk-ejection, fowl vasodepressor, antidiuretic, and vasopressor activities (see Tables). 2-*p*-Methoxyphenylalanine oxytocin, or O-methyl oxytocin (194), has practically no oxytocic or fowl vasodepressor activity.

Removal of the free amino group from the half-cystine in the 1-position of oxytocin has rather surprising effects (96). The resultant peptide, 1-desamino-oxytocin, has even greater potency on the rat uterus and in depressing the fowl blood pressure than does oxytocin itself. This is all the more extraordinary when one considers that covering this amino group by glycine, as in 1-glycyl oxytocin, essentially eliminates these activities (93). 1-Desamino lysine vasopressin has slightly less vasopressor activity than lysine vasopressin (96). These findings cannot be interpreted easily.

The specificity of the structure of the oxytocin molecule to the response of the rat uterus should not be stressed too heavily. Two synthetic straight-chain peptides that contain no cystine have been found to have activity on the rat uterus quantitatively quite comparable to that of oxytocin. These are bradykinin (48, 273) and angiotensinamide (250). Bradykinin (Arg·Pro·Pro·Gly·Phe·Ser·Pro·Phe·Arg) resembles oxytocin only in that it contains nine amino acid residues and shares two amino acids with oxytocin, proline and glycine. Angiotensinamide (Asp(NH_2)·Arg·Val·Tyr·Ileu·His·Pro·Phe) does contain a Tyr-Ileu component, as does oxytocin, as well as asparagine and proline residues. It has oxytocic activity estimated at 55 units/mg (250). It is certainly doubtful that the remote structural relationships between these peptides and oxytocin are of significance. It is worth noting, however, that these three extremely active peptides are very similar in molecular size.

B. Structures related to vasopressor activity

Vasopressor activities of a series of synthetic analogues have been estimated in several laboratories by intravenous injection into rats. In each instance they have been compared to standards containing arginine vasopressin. The results appear in Table 1. In a strict sense such assays are invalid since a cardinal rule of precise biological assay is that one must estimate the potency of an unknown preparation by comparison with a standard containing the same active principle. Nielsen (245) has analyzed rat vasopressor assays of lysine vasopressin against international standard (containing arginine vasopressin) in great detail. The error of such assays is increased over the error of similar assays of arginine vasopressin. The relative potency of lysine vasopressin determined in these assays varied with but slight changes in technique. Munsick (239) and Sawyer (288) have reported similar difficulties inherent in assaying dissimilar peptides on the rat uterus and on rat diuresis.

Although the errors involved in comparing unlike peptides may be great, the goal of the studies reported below is to distinguish rather gross alterations in pharmacological activities resulting from changes in molecular structure. The results must be considered rough comparisons relative to the standard beef pituitary powder, and not as precise assays adhering to the strict rules that are necessary if one is to establish statistical validity.

The presence of a highly basic amino acid in the 8-position appears important for vasopressor activity (180). Arginine vasopressin is the most potent neurohypophysial hormone. Lysine substituted in the 8-position as a less basic amino acid reduces the potency of arginine vasopressin or arginine vasotocin by about ⅓. Less basic 8-histidine or nonbasic 8-leucine reduces vasopressor activity even further. Removal of the amino group on the half-cystine in the 1-position of lysine vasopressin (desamino lysine vasopressin) also reduces its vasopressor activity. This suggests that a reduction of basicity of the entire molecule may also influence its pressor activity (96).

The presence of phenylalanine in the 3-position of the vasopressins appears somewhat less critical. 3-Isoleucine analogues, the vasotocins, have ⅕ to ½ the

TABLE 1

Rat vasopressor activity of neurohypophysial hormones and synthetic analogues

Analogue	Amino Acids in Positions				Activity, U/mg	References
	2	3	8	Other		
Arginine vasopressin	Tyr	Phe	Arg		300	242
Lysine vasopressin	Tyr	Phe	Lys		200, 270	242, 46
Desamino lysine vasopressin	Tyr	Phe	Lys	1-β-Mercaptopropionic acid	145	96
	Phe	Phe	Lys		50–60, 79	226, 47
Arginine vasotocin	Tyr	Ileu	Arg		64, 70, 125	242, 295, 180
Lysine vasotocin	Tyr	Ileu	Lys		39	47
	Phe	Ileu	Lys		32	46
	Tyr	Ileu	Val		9	46
Oxytocin (natural)	Tyr	Ileu	Leu		ca.7	242
Oxytocin (synthetic)	Tyr	Ileu	Leu		3, 4	295,* 36
Oxypressin	Tyr	Phe	Leu		3	47, 179, 242
Histidine vasopressin	Tyr	Phe	His		2, 1.5	181, 242
	Tyr	Tyr	Lys		1.6	47
Desamino oxytocin	Tyr	Ileu	Leu	1-β-Mercaptopropionic acid	1.2	96
	Phe	Phe	Leu		ca.0.9	47
	Tyr	Phe	Lys	9-Sarcosine	0.4–0.5	227
Desoxy oxytocin	Phe	Ileu	Leu		0.4	47, 295
	Tyr	Val	Leu		0.2	295,* 34
	Phe	Tyr	Lys		0.14	47
	Ser	Ileu	Lys		ca.0.1	46
	Tyr	Try	Lys		0.07	46
	Tyr	Tyr	Leu		ca.0.01	46
	Ser	His	Lys		<0.02	46
	Tyr	Ser	Lys		<0.01	46
	His	Ser	Lys		<0.01	46
	His	Phe	Lys		<0.01	46
	Phe	Tyr	Leu		<0.01	46
	Ser	Ileu	Leu		<0.01	46
	His	Phe	Leu		<0.01	46
Glycyl oxytocin	Tyr	Ileu	Leu	1-Glycyl	some	93
	Tyr	Leu	Leu		some	34
	Tyr	Try	Leu		?none	34
	Tyr	Ileu	Leu	5-Glu(NH_2)	?none	34
	Ser	Phe	Leu		?none	34
Isoasparagine oxytocin	Tyr	Ileu	Leu	5-*iso*Asp(NH_2)	?none	209
Isoglutamine oxytocin	Tyr	Ileu	Leu	4-*iso*Glu(NH_2)	inhibits	272
O-Methyl oxytocin	p-Methoxy-Phe	Ileu	Leu		inhibits	194

In Tables 1 through 7, values marked by an asterisk were obtained from assays on solutions containing uncertain concentrations of the active peptides. They were standardized in the reviewer's laboratory either by rat uterus or rat vasopressor assays. The absolute potencies (U/mg) indicated in the tables were calculated from the observed potencies of the solutions relative to estimated oxytocic or vasopressor potencies and the absolute oxytocic or vasopressor potencies of these peptides published elsewhere (34, 36, 47).

vasopressor activity of the respective vasopressins. Substitution of 3-tyrosine for 3-phenylalanine, representing but the addition of an hydroxyl group, however, reduces the vasopressor activity of lysine vasopressin by 99%.

Substitution of 2-phenylalanine for 2-tyrosine is compatible with vasopressor activity, although it is reduced about ⅔ in the case of 2-phenylalanine lysine vasopressin or oxypressin, and even more in the case of 2-phenylalanine oxytocin.

C. *Structures related to antidiuretic activity*

Arginine vasopressin, when injected intravenously into dogs or rats, is the most potent antidiuretic agent of the known neurohypophysial hormones or

analogues (347). The relative potencies of analogues of vasopressin (Table 2) depend to a great degree on the species in which they are compared (240, 244, 288, 337, 352).

The substitution of less basic 8-lysine for 8-arginine reduces the rat antidiuretic potency of lysine vasopressin to about 3/5 that of arginine vasopressin, and of lysine vasotocin to about 1/15 that of arginine vasotocin, when the maximal inhibition of urine flow is used as the measure of antidiuresis (288, 295). Substitution of a less basic amino acid such as 8-histidine or 8-leucine reduces the rat antidiuretic activity of vasopressin even further.

The duration of antidiuretic action is also markedly decreased by substitution of the 8-lysine moiety (244, 288, 295, 337). 8-Lysine analogues appear, therefore, to be even weaker when assayed by techniques that cannot detect transient changes in urine flow (333). It is extremely important in comparing antidiuretic activities to consider the details of the method and the criteria of antidiuretic activity used in calculating potency (244, 288).

Substitutions for the 3-phenylalanine do not result in such consistent changes. Arginine vasotocin (3-isoleucine arginine vasopressin) has about 1/4 the activity of arginine vasopressin on antidiuresis in the rat. Lysine vasotocin has about 1/15 to 1/30 the activity of lysine vasopressin.

The relationship of structural changes in the vasopressin molecule to the duration of antidiuretic action in rats requires further investigation before conclusions can be drawn. It is noteworthy that there are no comparable differences in the duration of the vasopressor effects of vasopressin analogues in the rat (295). The circulating half-life of arginine or lysine vasopressin in rats is about 1 minute (118, 324). The antidiuretic action of arginine vasopressin, in submaximal doses, usually lasts for 15 to 20 minutes (288). This indicates that the response persists

TABLE 2

Antidiuretic activities of neurohypophysial hormones and analogues in rats and dogs

Analogue	Amino Acids in Positions			Antidiuretic Activity			
				i.v. Rat		i.v. Dog	
	2	3	8	U/mg	Ref.	U/mg	Ref.
Arginine vasopressin	Tyr	Phe	Arg	300	288	300	347
Lysine vasopressin	Tyr	Phe	Lys	180	288	34	347
Arginine vasotocin	Tyr	Ileu	Arg	74	295	76	295
	Phe	Phe	Lys	29, 19	47, 295*	6	295*
Oxypressin (Sandoz)	Tyr	Phe	Leu	16	295*	21	295*
Oxypressin (du Vigneaud)	Tyr	Phe	Leu			10	295
Histidine vasopressin	Tyr	Phe	His			9	242
Lysine vasotocin	Tyr	Ileu	Lys	5	295*	2	295*
	Phe	Phe	Leu	5.7	47		
Oxytocin	Tyr	Ileu	Leu	1.2, 2.9	295*, 36	4	242
	Tyr	Ileu	Ileu	1.1	36		
	Tyr	Tyr	Lys	0.18	34		

See footnote to Table 1.

much longer than the circulating hormone. The antidiuresis produced by some 8-lysine analogues, however, may be only 5 to 10 minutes in duration (295). Since responses probably outlast the circulating hormones such differences in duration may reflect differences in the ability of various analogues to become fixed at the renal receptor site.

Vasopressin analogues have very different relative activities when assayed on dogs (Table 2) (295, 337, 352). Lysine vasopressin is only 1/7 as potent as arginine vasopressin. In this instance the difference in antidiuretic activity cannot be explained on the basis of duration of action. Continuous recording of urine flow and electrolyte excretion indicates that lysine vasopressin is less potent in reducing urine flow rate in the dog, irrespective of its duration of action (297).

Analogues containing an 8-lysine moiety have, in general, much weaker antidiuretic action than their respective 8-arginine analogues when assayed in dogs. Lysine vasotocin has only about 1/25 the dog antidiuretic potency of arginine vasotocin. 2-Phenylalanine, 8-lysine vasopressin is much less active in inhibiting diuresis in dogs than it is in rats. 8-Leucine analogues such as oxypressin or oxytocin, however, have comparable, if weak, antidiuretic potencies in rats and dogs.

Comparisons in other species yield even different orders of relative potency. In pigs, lysine vasopressin is at least as potent as, if not more potent than, arginine vasopressin in producing antidiuresis (240). This is an unusual example in which substitution of the less basic 8-lysine does not decrease activity compared to the corresponding 8-arginine analogue. In hens (242) and frogs (171, 344) arginine vasotocin is a much more potent antidiuretic agent than arginine vasopressin. In frogs arginine vasotocin is about three times as potent as lysine vasotocin, and much more active than oxytocin, arginine vasopressin, or lysine vasopressin (171).

In man the relative antidiuretic potencies of the vasopressins resemble those observed in dogs. Arginine vasopressin is approximately four times as potent as lysine vasopressin (87), and arginine vasopressin about 20 times as active as oxytocin or oxypressin on water diuresis (332). The 2-phenylalanine analogue of lysine vasopressin is only 1/20 to 1/40 as effective as an equal vasopressor dose of lysine vasopressin in inhibiting water diuresis in man (39a, 87).

D. Structures related to activity on the fowl oviduct

The use of strips from the "uterine" portion of the fowl oviduct was exploited as a pharmacological assay by Munsick *et al.* (242). Arginine in the 8-position is important for activity of analogues in causing contraction of such preparations (Table 3). 8-Lysine analogues of vasotocin or vasopressin are about 1/8 as active as the respective 8-arginine analogues. 3-Isoleucine analogues are 3 to 30 times more active than their 3-phenylalanine counterparts. The characteristics of this assay make it useful for the distinction of arginine from lysine vasopressin (147, 293, 301) and in the identification of arginine vasotocin (242, 293) in crude neurohypophysial extracts. The hen oviduct assay provided the first indication that arginine vasotocin was the pressor-antidiuretic principle in neurohypophysial extracts from nonmammalian vertebrates (241, 242, 353).

TABLE 3

Fowl oviduct activity of neurohypophysial hormones and analogues

Analogue	Amino Acids in Positions			Activity	Ref.
	2	3	8	U/mg	
Arginine vasotocin	Tyr	Ileu	Arg	640	242
Arginine vasopressin	Tyr	Phe	Arg	240	242
Lysine vasotocin	Tyr	Phe	Lys	75	295*
Oxytocin	Tyr	Ileu	Leu	29	242
Lysine vasopressin	Tyr	Phe	Lys	27	242
Oxypressin	Tyr	Phe	Leu	<1	242
Histidine vasopressin	Tyr	Phe	His	<0.2	242
	Phe	Phe	Lys	0.03	295*

See footnote to Table 1.

E. Structures related to activity on the rat uterus

"Oxytocic" activity can be assayed on uteri from rats or guinea pigs *in vitro* or on the uteri of several species *in vivo*. Relative potencies of neurohypophysial peptides determined by different oxytocic assays vary widely. The ionic composition of the medium in which isolated uteri are suspended and the reproductive status of the individual animal alter the sensitivity to the peptides, but not necessarily to all peptides equally (348). The rat uterus, suspended in a buffered magnesium-free Ringer's solution, is the most specific known assay for oxytocin (239). This assay is, therefore, used for the comparison of "oxytocic" activities shown in Table 4. The reader must remember that relative oxytocic potencies *in vitro* or *in vivo* in other species do not necessarily parallel the relative activities determined by this assay (34, 35, 239).

Substitutions of amino acids in the oxytocin molecule all reduce activity on the rat uterus in magnesium-free medium (Table 4). Removal of the free amino group on the N-terminal half-cystine, however, as in 1-desamino oxytocin, actually produces increased rat uterus activity. Substitution of an 8-isoleucine only decreases activity by $\frac{1}{3}$. 8-Valine oxytocin has approximately $\frac{1}{2}$ the activity of oxytocin. The 3-valine analogue is about $\frac{1}{7}$ as potent as oxytocin. 8-Arginine, 8-lysine, 2-phenylalanine, 3-phenylalanine, and 3-leucine analogues are somewhat less active. Arginine and lysine vasopressins have approximately $\frac{1}{50}$ and $\frac{1}{90}$ the activity of oxytocin. If, however, these analogues are compared to oxytocin by *in vivo* oxytocic assays (34, 35, 188), or on the rat uterus *in vitro* in the presence of magnesium (239), their relative potencies are increased several times.

Several analogues have been observed to be weak inhibitors of the action of oxytocin on the isolated rat uterus. 2-Phenylalanine, 8-lysine vasopressin, although possessing a weak intrinsic oxytocic action, inhibits the response of the rat uterus to oxytocin, added either simultaneously or subsequently (34, 294). 4-*Iso*glutamine and 5-*iso*asparagine analogues of oxytocin inhibit, as does the analogue with an extra tyrosine in the 2-position (34). Very high ratios of molar

TABLE 4

Rat uterus in vitro (no magnesium) activity of neurohypophysial hormones and analogues

Analogue	Amino Acids in Positions				Activity, U/mg	References
	2	3	8	Other		
Desamino oxytocin	Tyr	Ileu	Leu	1-β-Mercapto-propionic acid	698	96
Oxytocin	Tyr	Ileu	Leu		420, 415, 450	239, 36, 46
	Tyr	Ileu	Ileu		289	36
	Tyr	Ileu	Val		200	46
	Tyr	Val	Leu		59	34
Arginine vasotocin	Tyr	Ileu	Arg		37	239
Desoxy oxytocin	Phe	Ileu	Leu		30, 27, 32	44, 239, 47
Lysine vasotocin	Tyr	Ileu	Lys		20, 26	47, 295*
Oxypressin	Tyr	Phe	Leu		16, 20	239, 179
Arginine vasopressin	Tyr	Phe	Arg		9	239
Lysine vasopressin	Tyr	Phe	Lys		5	239
O-Methyl oxytocin	*p*-Methoxy-Phe	Ileu	Leu		*ca.* 5	194
	Tyr	Leu	Leu		*ca.* 5	46
	Phe	Phe	Leu		3.3	47
Oxytocin ring	Tyr	Ileu	—	7,8,9 missing	3.3	270
Histidine vasopressin	Tyr	Phe	His		1.5	181
	N-Methyl-Tyr	Ileu	Leu		1.2	46
	Phe	Ileu	Lys		1.0	46
	Tyr	Tyr	Leu		0.1	47
	Phe	Phe	Lys		<0.1 also inhibits	34, 294, 226
	Tyr	Try	Leu		0.04	46
	Tyr	Tyr	Lys		*ca.* 0.01	46
	Tyr	Ileu	Leu	1-Glycyl	some	93
	Phe	Tyr	Lys		<0.01	46
	Phe	Tyr	Leu		<0.01	46
	Ser	Ileu	Leu		<0.01	46
	His	Phe	Leu		<0.01	46
	Ser	His	Leu		<0.01	46
	Ser	Ileu	Lys		<0.01	46
	Tyr	Try	Lys		<0.01	46
	Ser	His	Lys		<0.01	46
	His	Phe	Lys		<0.01	46
	Tyr	Ser	Lys		<0.01	46
	His	Ser	Lys		<0.01	46
Desisoleucine oxytocin	Tyr	—	Leu		?none	45
	Tyr	Phe	Lys	9-Sarcosine	?none	227
	Tyr	Ileu	Leu	5-Glu(NH_2)	?none	34
	Tyr	Ileu-Ileu	Leu		?none	34
	Tyr-Tyr	Ileu	Leu		inhibits	34
Isoglutamine oxytocin	Tyr	Ileu	Leu	4-*iso*Glu(NH_2)	inhibits	272
Isoasparagine oxytocin	Tyr	Ileu	Leu	5-*iso*Asp(NH_2)	inhibits	209

concentrations of inhibitor to that of oxytocin are necessary in order to demonstrate such inhibition.

F. Structures related to milk-ejection activity

Oxytocin is the most effective of the peptides studied in causing milk ejection in lactating rabbits. This assay, however, does not discriminate as sharply as does the rat uterus against oxytocin analogues. 8-Isoleucine oxytocin is almost as active as oxytocin. 3-Valine oxytocin and 8-valine oxytocin are approximately ¾ as potent as oxytocin. 3-Leucine, 2-phenylalanine, and 3-phenylalanine oxy-

TABLE 5

Rabbit milk-ejection activity of neurohypophysial hormones and analogues

Analogue	Amino Acids in Positions				Activity, U/mg	References
	2	3	8	Other		
Oxytocin	Tyr	Ileu	Leu		360, 371, 450	242, 36, 36
	Tyr	Ileu	Ileu		328	36
	Tyr	Ileu	Val		310	46
	Tyr	Val	Leu		207, 240	47, 295*
Desoxy oxytocin	Phe	Ileu	Leu		60, 82, 141	44, 239, 47
Oxypressin	Tyr	Phe	Leu		60, 88	47, 239
Arginine vasotocin	Tyr	Ileu	Arg		80	242
Lysine vasotocin	Tyr	Ileu	Lys		55	47
Arginine vasopressin	Tyr	Phe	Arg		51	242
Lysine vasopressin	Tyr	Phe	Lys		51	242
	Tyr	Leu	Leu		ca. 45	46
	Phe	Phe	Leu		25	47
Histidine vasopressin	Tyr	Phe	His		18	239
	Phe	Ileu	Lys		12	46
	N-Methyl-Tyr	Ileu	Leu		3.1	46
	Phe	Phe	Lys		3	47
	Tyr	Tyr	Leu		1.5	47
Oxytocin ring	Tyr	Ileu	—	7,8,9 missing	ca. 1.1	270
	Tyr	Tyr	Lys		ca. 0.2	47
	Tyr	Try	Leu		0.1	46
	Tyr	Ser	Lys		0.04	46
	Ser	Ileu	Lys		ca. 0.01	46
	His	Phe	Lys		<0.01	46
	Ser	Ileu	Leu		<0.01	46
	Tyr	Try	Lys		<0.01	46
	His	Ser	Lys		<0.01	46
	Tyr	Ileu	Leu	5-Glu(NH_2)	some	34
	His	Phe	Leu		?some	34

See footnote to Table 1.

tocins are only slightly less active than 3-valine oxytocin (Table 5). Arginine vasotocin, lysine vasotocin, and even arginine and lysine vasopressins possess significant milk-ejection activity, 1/10 to 1/5 that of oxytocin. 2-Phenylalanine, 3-phenylalanine oxytocin, and 8-histidine vasopressin have milk-ejection potency considerably greater than any other activity for which they have been assayed.

The fact that the milk-ejection response of the rabbit shows much less structural specificity than the rat uterus limits its usefulness as an assay for oxytocin. It is, however, quite sensitive, and less liable to error due to the presence of agents in blood and tissue extracts that often interfere with rat uterus or fowl depressor assays. Arginine vasopressin has appreciable milk-ejection activity, and this assay is poorly suited for standardization of crude neurohypophysial extracts. Its intrinsic milk-ejection activity also raises the possibility that arginine vasopressin, as well as oxytocin, may play a role in the regulation of milk ejection in some animals.

G. Structures related to fowl vasodepressor activity

The fall in mean arterial pressure of the chicken (Table 6) is the official USP XVI method for standardizing posterior pituitary extracts and oxytocin solutions. It is, however, much less specific for oxytocin than is the rat uterus in

TABLE 6

Fowl vasodepressor activity of neurohypophysial hormones and analogues

Analogue	Amino Acids in Positions				Activity, U/mg	References
	2	3	8	Other		
Desamino oxytocin	Tyr	Ileu	Leu	1-β-Mercaptopropionic acid	578	96
	Tyr	Ileu	Ileu		498	36
Oxytocin	Tyr	Ileu	Leu		360, 432	242, 36
	Tyr	Ileu	Val		280	46
Arginine vasotocin	Tyr	Ileu	Arg		100, 75	242, 180
	Tyr	Val	Leu		58	34
Desoxy oxytocin	Phe	Ileu	Leu		60, 55, 63	44, 295,* 47
Oxypressin	Tyr	Phe	Leu		45, 55	179, 242
Lysine vasotocin	Tyr	Ileu	Lys		54, 45	47, 295*
Arginine vasopressin	Tyr	Phe	Arg		42	242
Lysine vasopressin	Tyr	Phe	Lys		28, 40	242, 46
Desamino-desoxy oxytocin	Phe	Ileu	Leu	1-β-Mercaptopropionic acid	*ca.* 14	96
	Phe	Ileu	Lys		*ca.* 8	46
	Tyr	Leu	Leu		*ca.* 7	46
	Phe	Phe	Leu		5.4	47
Histidine vasopressin	Tyr	Phe	His		5	242, 181
O-Methyl oxytocin	*p*-Methoxy-Phe	Ileu	Leu		*ca.* 5	194
	Phe	Phe	Lys		1.4, 0.5	47, 227
Glycyl oxytocin	Tyr	Ileu	Ileu	1-Glycyl	*ca.* 1, inhibits	93
	Tyr	Ileu	Leu	1-Mercaptoacetic acid	0.4–0.5	96
	N-Methyl-Tyr	Ileu	Leu		0.32	46
	Tyr	Try	Leu		*ca.* 0.1	46
	Tyr	Tyr	Lys		*ca.* 0.1	46
	Tyr	Try	Lys		0.08	46
	Ser	His	Leu		0.03	46
	Tyr	Tyr	Leu		*ca.* 0.03	46
	Ser	His	Lys		<0.02	46
	Tyr	Phe	Lys	9-Sarcosine	<0.01	227
	Ser	Ileu	Leu		<0.01	46
	Phe	Tyr	Leu		<0.01	46
	His	Phe	Leu		<0.01	46
	His	Phe	Lys		<0.01	46
	Tyr	Ser	Lys		<0.01	46
	Phe	Tyr	Lys		<0.01	46
	Ser	Ileu	Lys		<0.01	46

See footnote to Table 1.

magnesium-free medium, and is less precise. Since arginine vasopressin has significant fowl vasodepressor activity, the USP Standard Reference Powder or whole posterior pituitary extract contains more "oxytocic" units than does the oxytocin that it contains. There is, therefore, more oxytocin in one USP unit of oxytocin solution than in one USP unit of posterior pituitary extract. The fowl vasodepressor assay would thus appear to be an inadequate basis for standardization of oxytocin-containing solutions, and the use of the rat uterus assay, as in the BP, seems more appropriate.

The fowl vasodepressor response is unusual in that the two most potent peptides for its production are not known to occur naturally: desamino oxytocin (1-β-mercaptopropionic acid oxytocin) and 8-isoleucine oxytocin. Arginine vasotocin, believed to be the predominant neurohypophysial peptide in the chicken, has only about ¼ the vasodepressor activity of oxytocin. It does, however, ac-

count for the major portion of the total vasodepressor activity of fowl neurohypophysial extract (242). Both arginine and lysine vasopressins, as well as 3-valine oxytocin, desoxy oxytocin, lysine vasotocin, 3-leucine oxytocin, and desamino oxytocin, have appreciable fowl vasodepressor activities, ranging from about 1/8 to 1/20 that of oxytocin.

H. Structures related to activity on the frog bladder

It has long been recognized that responses of frogs, such as water uptake (139), reabsorption of bladder water (306), skin sodium transport (218), and antidiuresis (286), could be elicited more effectively by frog pituitary extracts than by mammalian extracts of equivalent oxytocic or vasopressor potency. This has been ascribed to the presence of an "amphibian water-balance principle" (139), or a "natriferin" (218), in extracts from neurohypophyses of amphibians and other cold-blooded vertebrates.

Water movement across the wall of frog bladders has been adapted as the basis of an assay, since it is a striking response to the "water-balance principle" which can be easily made quantitative (290). The assay shows extraordinary specificity for arginine vasotocin, which is almost certainly the "water-balance principle" or "natriferin" (7, 148, 171, 242, 290, 292, 293, 298). No analogue yet studied has more than 6% of the activity of arginine vasotocin by this assay (171, 290, 294). Analogues containing the unaltered oxytocin ring are most potent (Table 7); arginine vasopressin has less than 1% of the frog bladder activity of arginine vasotocin. 2-Phenylalanine is the only substituent in the oxytocin ring that is compatible with measurable activity. Substitution of 3-phenylalanine in arginine or lysine vasotocin, or in oxytocin, reduces activity to about 1% or less.

TABLE 7

Activity of neurohypophysial hormones and analogues on the permeability of the frog bladder in vitro

Analogue	Amino Acids in Positions			Activity, U/mg	
	2	3	8	*R. catesbiana* (290, 294)	*R. esculenta* (171)
Arginine vasotocin	Tyr	Ileu	Arg	10,000	14,000
	Tyr	Ileu	Ileu	800*	
Lysine vasotocin	Tyr	Ileu	Lys	380*	250
Oxytocin	Tyr	Ileu	Leu	360	450
Arginine vasopressin	Tyr	Phe	Arg	70	80
Desoxy oxytocin	Phe	Ileu	Leu	*ca.* 20*	
Oxypressin	Tyr	Phe	Leu	<20	
	Tyr	Val	Leu	<13*	
Histidine vasopressin	Tyr	Phe	His	<12	
	Phe	Phe	Lys	<6*	
Lysine vasopressin	Tyr	Phe	Lys	<5	12.5

See footnote to Table 1.

The unknown active principle in dogfish (*Squalus*) neurointermediate lobe extracts (259) has relatively high frog bladder activity (299, 302), suggesting the presence of the oxytocin ring. Although it is not oxytocin, arginine vasotocin or lysine vasotocin, it probably differs from these only in the nature of its 8-substituent (302).

V. RESPONSES TO NEUROHYPOPHYSIAL HORMONES

A. *Antidiuretic and related responses*

1. Responses of amphibian skin and bladder. Recent investigations on the amphibian skin and bladder provide the bases for current hypotheses concerning the action of antidiuretic hormones on the kidney (38, 195, 285, 286, 287, 290, 306, 323, 360, 364, 365). This work, therefore, appears to deserve consideration in detail.

Brunn (53) observed that neurohypophysial hormones increase water uptake by frogs kept in water. This is the "Brunn reaction" or "water-balance response" which occurs in skin bags (248) or in completely isolated fragments of skin (108, 282). The response results from an increase in the permeability of the skin to the movement of water following osmotic gradients. If frogs are placed in isosmotic or hyperosmotic solutions, water-uptake is prevented (282, 325).

The concept that neurohypophysial hormones cause an increase in the passive osmotic movement of water across the skin has been challenged on the basis of experiments on intact toads (27). In *Bufo marinus*, water-balance responses are usually very weak (294) and a major portion of the water uptake appears to be secondary to stimulation of sodium uptake (27). Capraro and Garampi (57) also observed that water movement across frog skin *in vitro* appears dependent largely on sodium movement. This observation may have resulted from peculiarities associated with season or species since it does not correspond to the experiences of others who have worked with isolated anuran skin (12, 217, 237, 282). Sodium transport does undoubtedly obligate some water movement. Most of the water movement in the presence of an osmotic gradient, however, can occur in the absence of sodium transport (217). Increased water movement in a direction opposite to net sodium transport has also been observed in skin exposed to hypertonic outside solutions (217). Such an increase in water movement cannot be easily attributed to primary sodium transport but probably represents decreased resistance to the osmotic movement of water across the skin.

The frog skin does not behave like an ideal homogeneous semipermeable membrane. In such a theoretical membrane the flux of water in both directions would increase in proportion to the increase in net osmotic flux. Water fluxes in both directions have been measured with heavy water. They do not increase in the presence of neurohypophysial hormones proportionately to the net water flux. Such observations led Capraro and Bernini (56) to suggest that active water transport was involved. Koefoed-Johnsen and Ussing (187), however, reasoning from similar observations, concluded that water movement in response to neurohypophysial hormones occurred by osmotic flow through minute pores. They suggested that the hormones dilate such pores. This would allow the flow of water

through channels. Such osmotic flow would not depend on a proportionate increase in unidirectional water fluxes.

Andersen and Ussing (12) have demonstrated, in direct support of the "pore theory," that neurohypophysial hormones alter the permeability of toad skin to acetamide and thiourea. Penetration of these small molecules across the skin is markedly enhanced by the addition of vasopressin. Osmotic movement of water exerts a "solvent drag" and enhances solute movement in the direction of water movement. These experiments indicate that both solvent and solute pass through the same channels and that these channels must increase in size or number under the influence of neurohypophysial hormones. Ussing (345) has studied the water-uptake of the skin itself. The findings indicate that neurohypophysial hormones act to increase the water permeability of the outward-facing surface of the epithelium. The pores, therefore, are believed to be on the outer surface of the basal layer of epithelial cells. Neurohypophysial hormones are only effective, however, if applied to the inner side of the skin.

Ewer (102) found that toads respond to neurohypophysial hormones by reabsorbing urine from the bladder. This suggested that the anuran bladder may respond in a manner similar to that of the skin. Experiments on bladders *in situ* demonstrated that these hormones affect water reabsorption across the wall of the bladder itself (306). Subsequently this response was demonstrated in bladders from several species *in vitro* (28, 171, 195, 219, 290). Toad and frog bladders are advantageous preparations for the study of the action of neurohypophysial hormones because of their extreme anatomical simplicity (196).

The isolated amphibian bladder responds in a manner quite similar to the skin. Water movement, and the penetration of urea or acetamide, are greatly accelerated in the presence of neurohypophysial hormones in both toad (219) and frog (290, 307) bladders. Water movement does not depend on sodium movement since it can occur in the absence of demonstrable sodium transport (195, 307) or in a direction opposite to net sodium transport (268, 290, 307). Neurohypophysial hormones appear to act to increase the permeability of the mucosal surface of the bladder (195). The hormones act, however, only if applied to the serosal surface (195, 290).

Neurohypophysial hormones also promote the active transport of sodium by frog skin or bladder (108, 171, 217, 218, 237, 290, 292, 346). For reasons that have been discussed, it is felt that this process is not essential for the action of these hormones on water movement. Although active uptake of sodium through the skin has adaptive value to amphibians the physiological importance of the increased transport in response to neurohypophysial hormones has not been established (285). The existence of an analogous response in the renal tubule has not been demonstrated.

The details of the cellular and molecular responses to neurohypophysial hormones remain largely unknown. Recent studies on amphibian bladders, however, suggest interesting hypotheses concerning the mechanisms by which these hormones act. Sodium transport by the toad bladder is accompanied by oxygen utilization, glycolysis, or both (197, 198). Anoxia markedly decreases, but does

not eliminate, the increase in sodium transport produced by the addition of vasopressin (196). The increase in permeability of the toad bladder to water or urea, however, under the influence of vasopressin is not accompanied by increased oxygen consumption (197, 268). Bentley (28) reported that lack of oxygen or glucose, or the addition of iodoacetate, cyanide, or 2,4-dinitrophenol inhibits the increase in water movement across the toad bladder in response to vasopressin. Rasmussen *et al.* (268) have not observed inhibition of this response by anoxia, lack of glucose, iodoacetamide, cyanide, 2,4-dinitrophenol, fluoroacetate or cold (2.5°C). They studied the response in choline-containing Ringer's solution so that sodium transport could not occur. It is likely that the response studied by Bentley (28) included water movement secondary to sodium transport and that it was this movement that was inhibited. The increase in water or urea permeability, presumably by the dilatation of pores, does not appear to depend directly on oxygen uptake, glycolysis, or phosphorylative oxidation, although these are necessary for the transport of sodium.

The effects on water transfer of changing the cationic composition of solutions bathing the serosal surface of the toad bladder, and the responses to neurohypophysial hormones have been investigated by Bentley (29). High calcium concentrations (9 mM/l) decrease the response to neurohypophysial hormones. Low calcium concentrations increase the resting water transfer across toad bladders and inhibit the response to neurohypophysial hormones (29). Magnesium, strontium, or manganese can partially substitute for calcium.

The effects of changing concentrations of univalent cations on the serosal side of the bladder have also been studied by Bentley (29). Either low or high potassium concentrations inhibit the response to vasopressin. Replacing 75% of the sodium with choline, in Bentley's (29) experiments, reduced the response to vasopressin to a much greater extent than other workers have observed in the same species (195, 268) or in the bullfrog (307) when virtually all the sodium was replaced with choline.

Rasmussen *et al.* (268) have investigated the effects of reagents that bind sulfhydryl groups on the action of vasopressin on water permeability of the toad bladder. N-Ethylmaleimide and several other sulfhydryl-binding reagents applied to either the serosal or mucosal surface can block the response to vasopressin. Iodoacetamide and several nonthiol-binding inhibitors are ineffective. If tritium-labelled vasopressin is added to the bladder it becomes bound to the tissue (316). This binding can be reversed with cysteine. N-Ethylmaleimide prevents binding. This is interpreted as indicating that vasopressin attaches to a receptor by a linkage involving interaction between the disulfide of the cystine moiety of the vasopressin molecule and sulfhydryl groups of the bladder. The site of such binding is not certain. The inhibitor applied to either surface can block the action of vasopressin applied to the serosal surface (268). Vasopressin is without effect when applied to the mucosal surface. This may imply that vasopressin attaches somewhere on the serosal side of a membrane that allows N-ethylmaleimide to pass but is impermeable to vasopressin.

It is difficult, however, to accept the interpretation that the inhibitor can

penetrate the mucosal surface of the bladder in quantities sufficient to block attachment of vasopressin. The mucosal surface of the bladder is only sparingly permeable to water or urea (195) and essentially impermeable to most larger molecules. It is probable that N-ethylmaleimide not only inhibits the attachment of vasopressin but also interferes directly with the response of the membrane. N-Ethylmaleimide added to either side of the membrane increases its water permeability (268, 316). Also, N-ethylmaleimide added after vasopressin does not reverse the response but prevents recovery after the vasopressin is removed. These phenomena suggest that this agent has effects on the bladder response other than those resulting from inhibition of the attachment of vasopressin to the receptor site.

2. Antidiuretic responses. The inhibition of water diuresis by neurohypophysial hormones in mammals is often accompanied by elevation of osmotic concentrations in the urine above that of plasma. Within the last few years, however, it has become evident that the production of hyperosmotic urine is not directly dependent on antidiuretic hormones (37, 38, 125, 286, 287, 321, 323, 364). Inhibition of water diuresis by neurohypophysial hormones ordinarily, but not necessarily, occurs before the urine is concentrated (37). Urinary concentrations hyperosmotic to plasma have never been observed in amphibians (285). Renal responses of frogs and toads to neurohypophysial hormones are uncomplicated by the presence of a concentrating operation. They are, therefore, useful in the study of the antidiuretic response *per se* (286, 287).

In either the frog or the toad neurohypophysial hormones can cause a striking increase in the tubular reabsorption of free water (286, 287). Urinary osmotic concentrations approach, but never exceed, that of plasma. The increase in the permeability of the renal tubule to the osmotic flow of water resembles the responses of the amphibian bladder and skin. It is logical to suggest that the responses of all three tissues to neurohypophysial hormones are essentially the same, probably the dilatation of pores allowing increased osmotic flow of water (306).

Whittembury *et al.* (360) have obtained evidence that neurohypophysial hormones can dilate pores in the amphibian kidney. The location of the pores is not, however, established. They found that the apparent pore size in *Necturus* kidney slices, gauged by the ability of small solutes to enter the slices *in vitro*, increases from 5.6 Å to 6.5 Å under the influence of vasopressin. Such a dilatation is consistent with the estimates of changes in pore size in skin or bladder in response to neurohypophysial hormones.

The antidiuretic response of the mammalian kidney has long been attributed by Smith (321) to increased permeability of the distal tubule to water. Wirz (364) obtained direct evidence supporting this hypothesis by collecting fluid from the distal convoluted tubules of rats by direct micropuncture. During water diuresis the urine throughout this segment is hypotonic. In the presence of antidiuretic hormone the urine is hypotonic at the beginning of the distal convolute, but becomes isotonic as it approaches the collecting duct. This convincingly demonstrates that the action of the antidiuretic hormone is to permit free water

to penetrate the distal tubular epithelium. In this respect the mammalian antidiuretic response appears identical with that in amphibians (286, 287).

The micropuncture experiments of Wirz (364) have been repeated and extended by Gottschalk (125) and his colleagues. They are in essential agreement as to the mechanism of the antidiuretic response. The question still remains as to whether segments other than the distal convolute are affected. It has been suggested that permeability of the collecting ducts to water also increases in antidiuresis (38, 125, 365). There is micropuncture evidence suggesting that the collecting duct as well as the distal tubule becomes more permeable to urea during antidiuresis (192). This is consistent with the hypothesis that the antidiuretic response derives from a dilatation of pores in those segments of the nephron distal to the site of free water formation.

Ginetzinsky (115) has described histochemical changes occurring in the distal tubules and collecting ducts that he believes reveal the action of antidiuretic hormones. Administration of vasopressin or dehydration causes a dissolution of the metachromatic intercellular substances between epithelial cells. This is believed to result in increased water permeability.

The identity of the intercellular substance has not been established; Ginetzinsky believes it contains hyaluronic acid since it disappears when treated with pneumococcal hyaluronidase. It does not occur in cyclostomes, teleosts or newborn rats (116). This is consistent with the lack of demonstrated antidiuretic responses in these forms (293). Adult rats, amphibians, and birds show antidiuretic responses and possess the metachromatic intercellular material that disappears under the influence of antidiuretic hormones (116). The phylogenetic and ontogenetic coincidence of this histochemical characteristic and known antidiuretic responses is interpreted as further evidence that they are related.

Ginetzinsky (115) also demonstrated "hyaluronidase" activity in the urine of dogs and rats associated with the antidiuretic action of vasopressin. Berlyne (39) has criticized Ginetzinsky's methods severely. He finds that there is "hyaluronidase" activity in dog urine but, if proper corrections are made for flow rate and electrolyte concentration, the activity is not correlated with antidiuresis to an appreciable degree. Similar experiments on conscious dogs that received vasopressin during water-mannitol diuresis have been performed by Kaplan *et al.* (177). "Hyaluronidase" activity was found to be directly proportional to urinary concentration and could not be correlated with vasopressin administration.

Testicular hyaluronidase in high concentrations does not influence the water permeability of toad (195) or frog (294) bladders.

In view of the lack of confirming evidence one must reserve judgment on the Ginetzinsky (115) hypothesis, attractive as it may sound. It is difficult to conceive of resynthesis of hydrolyzed intercellular hyaluronic acid occurring rapidly enough to account for the rate of recovery from water diuresis. In rats, complete recovery after maximal antidiuresis produced by intravenous lysine vasopressin (288) or some of its analogues (295) may take less than 5 minutes. Ginetzinsky (115, 117) has described the time course of the morphological changes that occur in the rat tubule after an unspecified dose of neurohypophysial extract. The effect

is maximal in 15 to 20 minutes. The slow onset of the response may result from subcutaneous administration. Ginetzinsky (115, 117) did not specify the route of administration. He has also described recovery of the intercellular substance. After water is administered to a previously dehydrated rat, regeneration is seen within 20 minutes and is complete in 1 hour. This morphological repair is approximately coincident with the appearance of water diuresis. It is unfortunate that observations have not been made during the rapid response and recovery observed after intravenous administration of lysine analogues of vasopressin. If the morphological changes could be shown to occur with comparable rapidity it would negate one argument against the Ginetzinsky hypothesis.

Fong *et al.* (106, 107) have studied the uptake of tritiated lysine and arginine vasopressins by the rat kidney. Activity is bound to kidney protein and can be released by incubation with cysteine. They suggested that vasopressin is bound in the kidney to dithiol groups on protein, similar to the binding observed in the toad bladder (316).

Darmady *et al.* (79) have studied the distribution of I^{131}-labelled lysine vasopressin (2-diiodotyrosine, 8-lysine vasopressin) in the rat kidney by autoradiography. The labelled derivative retained both antidiuretic and vasopressor activity. Labelled diiodotyrosine appeared in all segments. Labelled vasopressin appeared chiefly in the distal convoluted tubule and upper collecting duct. This agrees with the distribution one would predict on the basis of current belief concerning the location of antidiuretic action.

There are many conditions that may modify the renal antidiuretic response to neurohypophysial hormones. Strictly pathological conditions such as "nephrogenic diabetes insipidus" will not be discussed here.

The state of bodily hydration has an important influence on the antidiuretic response. Chronic forced hydration, produced by water administered with (203) or without (99) vasopressin, results in a decreased ability to reabsorb free water in response to supramaximal doses of vasopressin. Vasopressin chronically administered without simultaneous overhydration does not appear to have this effect (160). Inhibition of the antidiuretic response cannot be attributed to changes in filtration rate or urea excretion (99, 203). Bodily hydration appears to manifest itself by blocking the distal tubular response. Adrenal steroids may be involved either directly or by their effects on sodium and potassium distribution (203).

Hypokalemia decreases the ability of animals to form concentrated urine in response to neurohypophysial hormones (161). This appears to involve, at least in part, a decrease in the permeability of the distal tubule and collecting duct, even in the presence of vasopressin (114, 204, 220). The polyuria associated with desoxycorticosterone treatment or with hyperaldosteronism probably results largely from the effects of hypokalemia on the renal responses to vasopressin (114).

Hypercalcemia, produced by the infusion of calcium (24) or treatment with parathyroid extract (98) or vitamin D (221) also impairs renal concentrating ability and the response to vasopressin. Although the distribution of solutes in the renal papilla may be deranged, it appears that there is also interference with

the response of the distal tubule to antidiuretic hormones. Hypercalcemia can evoke the excretion of free water in acute experiments despite the continued infusion of maximally antidiuretic doses of vasopressin. It is unlikely that this could result from failure of the concentrating operation alone.

High calcium concentrations have been observed to inhibit the increase in apparent pore size that would be expected to occur in *Necturus* kidney slices treated with vasopressin (360). High calcium concentrations have also been reported to decrease the response of the isolated toad bladder to vasopressin (29). It is possible that calcium acts similarly on the mammalian kidney to prevent the increase in tubular permeability normally produced by vasopressin.

3. Saluretic responses. Neurohypophysial extracts have often been observed to enhance electrolyte excretion in experimental animals. The presence and magnitude of saluresis depends on the peptide, the species, and the pre-existing conditions of salt and water balance. Intravenous oxytocin and vasopressin can exert clear-cut saluretic effects in rats receiving infusions of NaCl solutions (169). If such a saluresis occurs in an animal that is already excreting a moderately concentrated urine, the urine flow increases. This is one well-defined mechanism by which neurohypophysial peptides can cause diuresis rather than antidiuresis (169, 283). Oxytocin can cause sufficient saluresis to exert a diuretic effect under conditions in which vasopressin is antidiuretic. In water-loaded subjects, however, oxytocin can usually produce a clear antidiuretic response if sufficient doses are used. This can occur without changes in filtration rate in the dog (60) as well as in man (1, 332).

Oxytocin, in doses that are not antidiuretic, can cause saluresis in dogs at low urine flows but not during water diuresis (52, 61). If such doses of oxytocin are, however, injected into a water-loaded dog receiving a sustained infusion of arginine vasopressin they are saluretic (61). This suggests that vasopressin must act on the kidney before oxytocin can produce saluresis (52). In one study, oxytocin was not saluretic in a dog with surgical diabetes insipidus unless vasopressin was also administered (52). Doses of oxytocin high enough to cause antidiuresis themselves are, however, saluretic in water-loaded dogs (60). This suggests that antidiuresis, not vasopressin *per se*, is the essential prerequisite for the saluretic action of oxytocin.

Brooks and Pickford (52) also observed in dogs that doses of oxytocin inadequate to produce saluresis when injected into a peripheral vein were effective if injected into the carotid artery. This has been confirmed by Chan (60), who demonstrated also a saluretic effect of oxytocin injected into the third cerebral ventricle of conscious dogs. These experiments suggest a central site of the saluretic action of oxytocin. Vasopressin, on the other hand, is no more effective in provoking saluresis when infused into the carotid artery than into a peripheral vein. Saluresis after vasopressin is usually brief, while that of oxytocin is much more prolonged. Vasopressin probably acts directly on the kidney to produce saluresis and antidiuresis (60). Oxytocin, however, has a central action that appears to evoke saluresis by an indirect mechanism, the details of which are completely unknown.

Saluretic responses to oxytocin and vasopressin have not been observed in man (1, 75, 235, 332). Their physiological significance, if any, in the dog or the rat remains to be established.

4. Actions on renal hemodynamics. Brooks and Pickford (52) reported that moderate doses of oxytocin increase renal plasma flow in dogs. This increase could be inhibited by small doses of vasopressin. Ali (11) also observed enhancement of glomerular filtration rate by oxytocin. Chan (60), however, has been unable to detect any consistent effect of oxytocin on renal plasma flow or glomerular filtration rate in conscious dogs. Abdul-Karim and Assali (1) observed no change in renal plasma flow or glomerular filtration rate in men infused with synthetic oxytocin in a wide range of doses. Cross *et al.* (75) observed either no change or a fall in filtration rate and renal plasma flow during oxytocin infusions. Mertz (235) has, however, observed increased *p*-aminohippurate clearances in human subjects after large doses of oxytocin, although other subjects in the same series showed depressed renal plasma flow.

A role of oxytocin in the maintenance of renal function in dogs has been inferred by Demunbrun *et al.* (82) on the basis of observations on animals with surgical diabetes insipidus. The failure of Brooks and Pickford (52) to observe depressed renal function in diabetes insipidus dogs, and the lack of consistent effects of oxytocin on renal hemodynamics in Chan's (60) experiments fail to support this hypothesis.

B. Oxytocic responses and milk ejection

Responses of mammalian uteri to neurohypophysial hormones have been discussed extensively in the contributions to the 1959 symposium on oxytocin held in Montevideo (55) and will not receive detailed consideration here.

The sensitivity of the uterus to neurohypophysial peptides depends on many factors (348). Species, reproductive status, and the ionic milieu are important modifying influences. The role of progesterone in suppressing uterine reactivity is subject to considerable controversy. Differing opinions derive largely from experiences with different experimental animals. The rabbit is the classical example of a mammal in which progesterone renders the myometrium refractory to the oxytocic action of neurohypophysial peptides (77, 314). In human females, huge doses of progesterone do not appear to decrease the sensitivity of the uterus to oxytocin or to delay the onset of spontaneous labor (265). The human uterus becomes quite responsive to oxytocin early in the last trimester when endogenous progesterone levels are high (55a, 238). There does not appear to be aı abrupt increase in sensitivity near term. The rabbit uterus, on the other hand, becomes responsive only in the last few days of pregnancy (77, 314).

Estrogens appear generally to increase the sensitivity of the uterus to oxytocin (77, 314, 348). This increase is inhibited by progesterone in the rabbit, but not, apparently, in several other mammals, including human females.

Neurohypophysial peptides appear to exert their characteristic effects on uterine smooth muscle by acting on the surface of the muscle fibers (77, 100, 176). The details of their actions are certainly not clear. Depolarization of the

muscle cell membrane by the application of potassium does not appear to inhibit the response of the rat uterus to oxytocin (100). In the rabbit uterus, however, depolarization reduces the response to oxytocin and changes its character (77). Oxytocin, in moderate doses, appears to increase the intrinsic motility of the uterus, to lower the threshold to electrical stimulation (77), to lower the membrane potential, and to slow conduction velocity (176). The result is a slow, rhythmic, coordinated contraction. Such rhythmic contractions are accompanied by trains of action potentials. Higher doses tend to produce sustained contraction, either *in vitro* (77, 176) or *in situ* (266); this is associated with depolarization of the muscle cell and the absence of propagated action potentials (176). These characteristics of the uterine response to oxytocin indicate that it is extremely important that minimally effective doses be sought and used in the management of labor.

Oxytocin is an extremely potent stimulator of the mammalian uterus and is undoubtedly secreted during labor (105). It is likely that endogenous oxytocin exerts a significant influence on uterine motility during parturition, although it is not clear just how important it may be in the initiation and maintenance of labor. Neurohypophysial hormones may also participate in the process of egg-laying in birds and reptiles. Arginine vasotocin is very effective in causing contraction of the isolated hen or turtle oviduct (242). Whether neurohypophysial hormones have any comparable actions in vertebrates other than the amniotes remains to be established (293).

The milk-ejection response resembles the oxytocic response in some respects. Both actions of neurohypophysial peptides are on contractile elements. In the mammary gland the responsive units are not smooth muscle but the myoepithelial cells that surround the alveoli and smaller ducts. The physiology of these cells has been recently reviewed by Linzell (207). Contraction of alveoli can be directly observed after the addition of oxytocin (207), and strips of mammary tissue suspended *in vitro* contract under the influence of oxytocin (233). The only contractile elements present that could reasonably be expected to produce these effects are the myoepithelial cells.

Contraction of the alveoli forces milk from alveoli into the ducts, raises the intraductile pressure and produces milk "let-down," or, more properly, "milk ejection." This is necessary if previously secreted milk stored within the alveoli is to be made available for milking or suckling.

Single intravenous doses of oxytocin, as little as 1 mU, cause an abrupt and transient rise in intramammary pressure in the rabbit (349) and the human female (54). Sustained infusion or intramuscular or intranasal administration can produce rhythmic waves of increased pressure or a sustained "tonic" elevation in the rabbit (33). In these respects the patterns of response resemble those seen in the uterus.

C. *Vascular responses*

The first described pharmacological property of neurohypophysial extracts was their ability to raise the blood pressure in anesthetized animals (249). The vasopressor response remains a useful criterion for the biological standardization

of vasopressin solutions. It is doubtful, however, that this response is of physiological importance in the regulation of cardiovascular function.

Vasopressor responses to large doses of vasopressin can be observed in intact, unanesthetized animals. The necessity for using large doses probably derives largely from rapid compensation by autonomic reflexes. For this reason, in biological assays pithing, decerebration, or adrenergic blocking drugs are employed in addition to deep anesthesia to enhance sensitivity to the vasopressor peptides. Under such circumstances, vasopressor responses may be observed with doses that are not entirely out of the range of possible physiological secretion, often only about 10 to 50 times the doses that are required to cause antidiuresis.

In normal men large intravenous doses of vasopressin produce a slight increase in arterial pressure (183, 355). This returns to resting levels even if infusion is continued. The pressor response appears to result primarily from peripheral vasoconstriction, since it is accompanied by pallor and a sharp reduction of blood flow to the hand. During continued infusion hand blood flow returns to resting values while blood flow to the whole forearm increases markedly (183). This compensatory response occurs even after the nerves to the forearm are blocked. The mechanism certainly is not clear.

Patients with "primary autonomic insufficiency," characterized by orthostatic hypotension, anhidrosis and impotence, show clear vasopressor responses. Reduction of sympathetic vasoconstrictor tone, therefore, appears to allow the peripheral vascular response to vasopressin to manifest itself as a sustained rise in systemic arterial pressure. Vasopressin in such subjects increases peripheral resistance without altering cardiac output (355) although it has been known for many years that sufficient doses can cause coronary vasoconstriction (356).

Large doses of oxytocin (185, 263) or 3-valine oxytocin (184) administered intravenously to normal subjects can cause an increase in blood flow to the hand and forearm. This increase subsides despite continued infusion. There may also be a transient fall in blood pressure. Cardiac output rises, probably in response to the fall in peripheral resistance (185). Wagner and Braunwald (355) did not note any consistent change in blood pressure in normal subjects injected with large doses of oxytocin. In their patients with autonomic insufficiency, however, infusion of oxytocin provoked transient vasodepression followed by slight hypertension. Thomson (332) did not observe any change in blood pressure in normal subjects receiving oxytocin. Caldeyro-Barcia (54) reported that 1 to 8 units/min of oxytocin infused intravenously into pregnant women caused a transient fall in blood pressure followed by a rise that persisted for the duration of the infusion.

When oxytocin is injected into anesthetized rats pretreated with an adrenergic blocking drug, as in routine vasopressor assays (291), it produces a brief vasopressor response that resembles closely the response to a much smaller dose of vasopressin (348).

Oxytocin has been observed to have little or no vasopressor effect in castrated or diestrous female, or male rats (208, 263). These rats were anesthetized but had not received an adrenergic blocking drug. In rats in estrus, or with stil-

bestrol treatment, oxytocin had a pressor effect (208). There does not, however, appear to be any marked difference in the responses of male or estrous female rats pretreated with phenoxybenzamine (Dibenzyline) (294); oxytocin elicits clear vasopressor responses in both. This further emphasizes that autonomic influences may determine the nature of the net cardiovascular response to a neurohypophysial peptide.

In most birds oxytocin, arginine vasotocin, and vasopressin produce predominantly vasodepressor responses. Vasopressin and arginine vasotocin may produce a vasopressor response as well, usually following a brief depressor phase. The physiological significance of these responses, if any, is certainly not known. The vasodepressor response is the basis, however, for the standard USP assay for oxytocin. It can occur in the perfused fowl leg and, therefore, probably results from peripheral vasodilatation (368). Studies on vascular responses in reptiles and amphibians (305, 367) have not yielded information supporting the viewpoint that neurohypophysial hormones have any consistent or important role in cardiovascular regulation. Large doses elicit variable responses in different species or in different individuals of the same species. Responses may be predominantly vasopressor, vasodepressor, or diphasic and are usually relatively weak (305, 367).

D. Release of adenohypophysial hormones

The responses to neurohypophysial principles discussed in preceding sections are by peripheral organs, including the renal tubules, blood vessels, uterus, and mammary glands; they respond to principles present in the general circulation. Neurosecretory products are believed to be released also into the hypophysial portal system from the median eminence. These products could reach the adenohypophysis in high concentrations and might constitute an important link in the central nervous control over adenohypophysial secretion.

The median eminence and the neural lobe have evolved as specialized portions of a more primitive, undivided neurohypophysis (126, 127, 173, 362, 363). In cyclostomes and most bony fishes the neurohypophysis is a single organ closely applied to adenohypophysial tissue or actually embedded within it. There is no true hypophysial portal system and no neural lobe. Neurosecretory products of hypothalamic nuclei accumulate in endings within the neurohypophysis and are presumably released directly into the adenohypophysis and its blood supply.

The neural lobe appears in a primitive form in certain lungfish (363) and is well developed in terrestrial tetrapods (126). It is a posterior and dorsal extension of the neurohypophysis which acquires a circulation distinct from that of the median eminence and adenohypophysis. The phyletic coincidence of the appearance of the neural lobe, terrestrial habitat, and known antidiuretic responses implies that they are related. The diversion of the effluent veins of the neural lobe to discharge into the general circulation indicates that this lobe serves as an outlet for the systemic release of hormones. While in tetrapods the neural lobe serves as a site for the release of antidiuretic hormones (173, 300), the neurohypophysis of cyclostomes or teleosts releases no hormones known to be

antidiuretic in these forms. Rather, the total neurohypophysis in these fishes, like the median eminence in tetrapods, is believed to release mediators that regulate adenohypophysial function (126, 127, 173, 362, 363).

Destruction of the median eminence, interruption of the hypophysial portal veins, or transplantation of the adenohypophysis to locations remote from the median eminence all interfere with adenohypophysial secretion (134). The failure of normal ACTH release following lesions in or near the median eminence has been observed repeatedly (109, 110, 134, 168, 210). Since the median eminence and the neural lobe have common phyletic and embryological origins, it is natural that neurohypophysial preparations have been studied with respect to their actions on the release of ACTH.

Many reports demonstrate that neurohypophysial extracts and natural vasopressins can elicit ACTH secretion in intact animals or after lesions of the median eminence (130, 168, 210, 213, 222). Martini *et al.* (59, 222), demonstrated ACTH release from adenohypophysial grafts in the anterior chamber of the rat eye when natural vasopressin was injected directly into the anterior chamber. Synthetic lysine vasopressin can undoubtedly cause ACTH release (174, 175, 212, 216). ACTH release in response to natural vasopressin preparations cannot, therefore, be attributed solely to nonvasopressin contaminants. Large doses of lysine vasopressin are usually necessary to release ACTH unless the peptide is injected into an eye containing an adenohypophysial graft (59, 175, 224).

Very small doses of highly purified arginine vasopressin injected into the third ventricle of conscious dogs cause enhancement of adrenal 17-hydroxycorticosteroid secretion (190); equimolar doses of arginine vasotocin, oxypressin, and oxytocin do not have this effect. An intraventricular injection of arginine vasopressin does not insure that it exerts a direct action on the adenohypophysis. It does show, however, that "physiological" quantities (2 mU or about 7 mμg) can trigger significant ACTH release when applied in this manner. The potency of intraventricular arginine vasopressin in inducing ACTH release in dogs raises the possibility that it may be a physiological releaser in this species. The dog hypothalamus contains much higher concentrations of arginine vasopressin than are found in hypothalami from the other mammals that have been studied (350). This store of hypothalamic vasopressin could possibly act on ACTH release, a function that may be served by other peptides in other species.

Nichols and Guillemin (243) estimated that it is necessary to inject 3,000 to 7,000 times as much lysine vasopressin into a peripheral vein of a dog to produce ACTH release as is required for maximal antidiuresis. The effectiveness of intraventricular arginine vasopressin in dogs, however, counters the conclusion of these authors that vasopressins release ACTH only in pharmacological doses. It is unfortunate that we do not have comparative information on the releasing activity of intraventricularly administered lysine vasopressin.

Large doses of vasopressins have been reported to enhance adrenal corticoid secretion in hypophysectomized animals (156, 168, 277). This may result from release of ACTH from peripheral storage sites (277), or from direct stimulation

of cortical release of steroids by the vasopressins (156, 168). The striking effectiveness of arginine vasopressin injected into the third ventricle in Kwaan and Bartelstone's (190) experiments certainly established that this peptide has a potent central ACTH-releasing action at doses far below those required to elicit peripheral responses.

Release of ACTH from fragments of rat pituitary incubated *in vitro* has been employed by Saffran *et al.* (280) and Guillemin *et al.* (131) as a bioassay to guide attempts at isolation of the factor(s) responsible. Potent preparations that are not vasopressins have been separated by chromatography from hypothalamic neurohypophysial extracts. These have been called "Fraction D" (131) and "corticotropin-releasing factor" (CRF) (280).

The specificity of CRF assays *in vitro* has been questioned. Although criticism may be justified in certain respects, these assays have made possible the preparation of extracts of high CRF potency *in vivo* as well as *in vitro*. Such preparations have CRF activity far greater than do equipressor amounts of vasopressin. This has been clearly demonstrated by determining changes in corticosterone secretion in rats with hypothalamic lesions (213) or under morphine-pentobarbital anesthesia (130). Martini *et al.* (224), however, found their CRF preparation to be no more potent in causing ACTH release by pituitary grafts in the anterior chamber than equipressor doses of lysine vasopressin. They followed adrenal ascorbic acid depletion as an index of ACTH release. This method has not, in the hands of other workers (130, 213), proved as sensitive or discriminating as an index of CRF activity as have plasma corticosteroid determinations. At present, the available evidence favors the view that there is activity in extracts of neurohypophysis and hypothalamus which is not due to vasopressin and which is extremely effective in releasing ACTH (279, 308).

Schally *et al.* (281, 310) have obtained highly purified CRF preparations that yield, on hydrolysis, all the amino acids of arginine and lysine vasopressins as well as alanine, serine, and histidine. Thioglycollate destroys CRF activity, indicating that the S–S group of cystine may be important to activity. These observations certainly indicate a molecular resemblance between CRF and the vasopressins and may explain why good CRF preparations have some residual vasopressor activity. Similarly, the CRF activity of the vasopressins may derive from a structural resemblance to the true CRF's (173, 279). Such overlapping activities can be compared to the observation that vasopressin has some inherent oxytocic activity, while oxytocin has weak vasopressor activity.

The evidence that CRF activity may reside in vasopressin-like peptides makes the determination of CRF potencies of synthetic vasopressin analogues of considerable interest. Boissonnas (46a) has reported on *in vivo* assays of a few analogues, performed by the technique of Guillemin *et al.* (130). A preparation of Schally's CRF (132, 310) has about 10,000 CRF units/mg, a unit of activity being taken as that of 10 μg of Guillemin's "Fraction D" (131). This CRF preparation also has about 200 vasopressor units/mg. The CRF/vasopressor ratio is therefore about 50. Lysine vasopressin has a CRF/VP ratio of 15 and about 4,000 CRF units/mg. Several analogues show CRF/VP ratios significantly higher than lysine vasopressin, although their CRF activity is comparatively

weak (5 to 20 U/mg). The high CRF ratios derive, therefore, in large part from their extremely weak vasopressor activities. These analogues are 2-serine, 8-lysine oxytocin, 2-serine, 3-histidine, 8-lysine oxytocin, and 3-serine, 8-lysine oxytocin. Although these analogues are far too weak to be responsible for the CRF activity of Fraction D or Schally's preparations, the high CRF/VP ratios are of interest as offering at least a clue to the molecular configurations that favor CRF activity over vasopressor activity. It is also worth noting that these are all serine-containing analogues, and serine was present in the CRF preparations analyzed by Schally *et al.* (310).

Guillemin *et al.* (132) have separated two peaks of CRF activity by countercurrent distribution of pig neurohypophysial extracts. One moves with one of the melanophore stimulating hormones (α-MSH), and has been called α-CRF; the authors believe that it resembles the vasopressin-free CRF isolated by Gros and Garilhe (129) by countercurrent separation. The second is closely associated with lysine vasopressin. The α-CRF can be separated from α-MSH by chromatography. It contains the amino acid sequence Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Try-Gly-Lys-Pro-Val-Thr-Ala-Leu. This is essentially α-MSH with the addition of three amino acids, threonine, alanine, and leucine. It has a heptapeptide sequence that also appears in ACTH. It is not surprising, therefore, that this α-CRF exhibits both MSH and ACTH activities. Kappeler and Schwyzer (178) have reported that the heptapeptide, Met-Glu(NH_2)-His-Phe-Arg-Try-GlyOH, is the most active of several synthetic peptides related to α-MSH when assayed for *in vitro* CRF activity (81). This peptide is actually the heptapeptide sequence common to ACTH and α-MSH. It is possible that α-CRF is an intermediate in the synthesis of ACTH (132). The presence of such an intermediate in neurohypophysial extracts does not indicate that it is necessarily of neurohypophysial or hypothalamic origin. Posterior lobes have been observed to contain relatively large quantities of both MSH and ACTH (236, 274) which are probably of adenohypophysial origin. α-CRF may also originate in the adenohypophysis. Whether it acts as a true tropic hormone influencing ACTH secretion is certainly not established.

Guillemin *et al.* (132) have named the vasopressin-like CRF fraction β-CRF. This resembles the peptide studied by Schally *et al.* (310) and contains cystine. It does not have appreciable ACTH or MSH activity, but does have 50 to 100 times the CRF activity of α-CRF *in vitro* or *in vivo*. It would appear a more likely candidate as the long-postulated physiological CRF of hypothalamic origin. Much more evidence is necessary before this can be established.

Rates of secretion of adenohypophysial hormones other than ACTH are probably influenced also by factors released by the median eminence into the hypophysial portal system. The evidence concerning this has been reviewed recently by others (101, 134, 135, 136, 137, 327). In no instance has the identity of the controlling principle or principles been determined.

Thyrotropin secretion is regulated in part by the hypothalamus (134, 136, 137). Large doses of lysine vasopressin and oxytocin, however, do not appear to have a direct thyrotropin-releasing effect on the adenohypophysis (76).

The situation concerning gonadotropin release is rather obscure (101). Trans-

plantation of the pituitary to the renal capsule of rats appears to produce suppression of secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Secretion is restored if the transplant is successfully regrafted beneath the median eminence (247). The mediator of the tropic influence of the median eminence is not known. That oxytocin may be capable of modifying gonadotropin secretion is suggested by the observation that natural oxytocin shortens the diestrous period of cows (14). This may, however, be a response to a contaminant of the natural oxytocin solution used and is not necessarily a direct response of the adenohypophysis. Martini *et al.* (225) reported that synthetic oxytocin and lysine vasopressin, when administered to rabbits in large doses, increase urinary gonadotropin excretion. Activity in the urine was assayed by injection into immature mice and determination of the increase in uterine weight. The specificity of this apparent increase in gonadotropin output is not clear, since many stimuli can induce an adenohypophysial response in rabbits as indicated by ovulation. The synthetic peptides do appear capable of affecting LH release in rats. McCann *et al.* (215) found that Pitressin can stimulate LH release as estimated by the ovarian ascorbic acid depletion method in immature rats. This effect persisted, however, after hypophysectomy, indicating that it was not solely due to adenohypophysial stimulation. Extracts of the median eminence and pituitary stalk produced a greater release of LH but were ineffective in hypophysectomized animals. In view of the present evidence one must reserve judgment on whether there is a specific LH-releasing factor secreted by the median eminence, and whether oxytocin or vasopressin can produce gonadotropin release by direct action on the adenohypophysis.

Benson and Folley (26) have suggested that oxytocin released during suckling stimulates release of adenohypophysial luteotropin (LTH, prolactin, lactogenic hormone). Such a mechanism might explain why nursing prolongs lactation. These authors observed that oxytocin injections could delay mammary involution in nursing rats after removal of their litters. McCann *et al.* (214) also found that oxytocin can delay mammary involution in lactating rats bearing hypothalamic lesions that would otherwise inhibit LTH secretion. According to Desclin (84) and Stutinsky (326) pseudopregnancy and the formation of deciduomata in rats appear after treatment with oxytocin. This finding also implies release of LTH. Several authors (62, 215, 276), however, have failed to produce pseudopregnancy even after massive subcutaneous or intraperitoneal doses of oxytocin, vasopressin, or whole posterior pituitary extract into proestrous or estrous rats.

The assumption that the median eminence exerts a positive LTH-releasing effect is certainly not supported by experiments on pituitary transplants. Certain lesions of the hypothalamus and median eminence (138, 211) enhance LTH secretion. Transplantation to the renal capsule also increases LTH secretion by the pituitary (84, 229, 246, 247, 267). This suggests that the hypothalamic influence on LTH secretion is predominantly suppressive. It is still possible that oxytocin can promote LTH-release either by direct stimulation or by antagonizing the secretion or the action of an inhibitory factor. Release of LTH, however,

often appears to be a nonspecific response. Meites *et al.* (228, 231, 232) reported that a large variety of pharmacological agents and tissue extracts from the hypothalamus and cerebral cortex can initiate lactation in properly primed rats. They (230) have also suggested that oxytocin may prolong lactation after removal of the litter by directly stimulating the mammary glands. Rothchild and Quilligan (276) also criticized severely the evidence that oxytocin can produce pseudopregnancy. In view of the confusion surrounding the subject of LTH-releasing influences, we must be cautious in suggesting that neurohypophysial principles, either known or unknown, may participate directly or indirectly. This possibility, however, merits further investigation.

E. Responses of the central nervous system

Scattered observations indicate that neurohypophysial principles may act at central sites other than the adenohypophysis. Neurosecretory fibers have been found that terminate in parts of the brain distant from the median eminence or neural lobe (23, 200, 313). Activities resembling those of neurohypophysial peptides have also been reported in the brain. Extracts of the subcommissural organ were reported to be antidiuretic when injected intravenously into rats (251). In earlier experiments the reviewer was unable to demonstrate such activity in extracts of rat subcommissural organs (296). The roof of the third ventricle of toads (*Bufo arenarum*) that includes the choroid plexus has been reported to inhibit diuresis when injected intravenously into rats (339, 343). The total activity per toad was approximately 1 milliunit (not 1 unit as misprinted in the original paper). The reviewer also assayed this portion of the brain in *Bufo arenarum* and in *Rana catesbiana*. Antidiuretic activity was greater than in most other portions of the brain but the concentration was only about 1/1000 that in the neurohypophysis. In the absence of evidence bearing on the specific nature of this antidiuretic activity, one cannot place much confidence in the belief that it is due to a neurohypophysial peptide.

Responses to neurohypophysial peptides suggest that there may be receptors within the central nervous system. The evidence that oxytocin acts at an intracranial site in provoking natriuresis in dogs (52, 60) has been discussed in a previous section. Localized electrical activity in the posterior hypothalamus occurs after intravenous injection of large doses of Pitressin (330). The meaning of this is not clear, and it has not been established that it represents a direct response. Vasopressin in large doses has also been found to delay the onset of drinking in dogs made thirsty by the intravenous administration of hypertonic sodium chloride solution (25, 162) and to inhibit thirst in patients with diabetes insipidus (257) when water is withheld. Very large doses of oxytocin or vasopressin provoke the "spawning reflex" in certain fishes (97, 361). This effect is not mediated by the adenohypophysis or gonads since it persists after hypophysectomy and gonadectomy. None of these responses have been conclusively shown to be direct actions on the brain, but they do suggest that we should remain aware of the possibility.

VI. GENERAL CONCLUSIONS

The recent description of the chemical structures of the natural neurohypophysial hormones has been a tremendous stimulus to research on the active peptides. Knowledge of their molecular nature has made possible more penetrating studies of the manner in which they are formed within the hypothalamus, stored in the neural lobe, and released in response to appropriate stimuli. The relations between the hormones and the proteins with which they are associated have been at least partially clarified. The enzymatic processes involved in the metabolism of the hormones in blood and tissues have been studied in ways that promise more exact knowledge of their physiologic disposition. The rates at which the hormones are removed from the circulation and the sites of inactivation and excretion are now better understood.

Synthesis of neurohypophysial hormones and their structural analogues has supplied invaluable material for studies on structure-activity relationships. New peptides may be forthcoming with substantial advantages over existing preparations not only to the investigator but perhaps also to the therapist. Synthetic peptides have provided preparations of known purity which have facilitated detailed studies of the mode of action of neurohypophysial hormones.

Recent years have also seen advances in our yet meager understanding of the role of neurohypophysial peptides in the control of adenohypophysial function. This is an area in which we can reasonably expect significant new findings in the next few years.

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