Antidiuretic Hormones

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There are at least 3 antidiuretic hormones. Arginine vasopressin is the antidiuretic hormone of most mammals, including man. This function is served by lysine vasopressin in the pig, and by arginine vasotocin in birds, reptiles and amphibia.

All 3 hormones are closely related chemically and act on the kidney in a similar manner. Arginine vasotocin also exists in fish, in which the antidiuretic response is not known to occur. This antidiuretic hormone appeared, therefore, in vertebrate phylogeny before it acquired antidiuretic function. Anatomic considerations suggest that the fish neurohypophysis serves to regulate adenohypophysial secretion. There is evidence that such a primitive type of neurohypophysial function has persisted throughout subsequent vertebrate evolution.

The antidiuretic hormone of man has been chemically identified as arginine vasopressin. This is not, however, the antidiuretic hormone of pigs, birds, reptiles or amphibia.

Antidiuresis occurs in these animals in response to neurohypophysial peptides that differ chemically from arginine vasopressin. From a comparative viewpoint, therefore, there is no single antidiuretic hormone but several antidiuretic hormones. All appear closely related chemically and act on the kidney in an analogous manner. An examination of the chemical relationships of these neurohypophysial antidiuretic hormones and their evolution should be of interest and may offer additional insight into their physiologic functions.

Antidiuretic hormones are formed by neurosecretory cells within the hypothalamus and are transported along their axons to be stored in dilated nerve endings closely applied to the capillaries of the neurohypophysis. The appear to be stored in loose combination with protein. The chemical form in which antidiuretic hormones are released is not clear. Antidiuretic activity, however, certainly resides in octapeptides such as the vasopressins and their analogues.

The best-defined action of antidiuretic hormones is the promotion of renal tubular reabsorption of free water. This action is of major physiologic importance to mammals and probably to nonmammalian terrestrial tetrapods. It is the basic step in the renal mechanism for the conservation of water. Suppression of antidiuretic hormone secretion is, conversely, essential for the production of water diuresis and the elimination of excess water.

Changes in osmotic concentration of blood reaching osmoreceptors within the brain influence the release of antidiuretic hormones in a manner that tends to restore and maintain a stable plasma osmotic pressure. The volume of circulating blood also appears to modify release independently of changes in osmotic pressure in such a manner that water is excreted or retained to restore a normal circulatory volume. Pain, emotion and drugs are among many other factors that may stimulate the release of antidiuretic hormones.

Neurohypophysial Structure and Function in Nonmammalian Vertebrates

Neurohypophysial hormones can cause antidiuresis in mammals, birds, reptiles and amphibia. A similar action in fish, however, has never been demonstrated. The antidiuretic response appears to be an adaptation re-

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Highly schematic diagrams of the hypophysial circulation in 4 vertebrate types. Solid arrows represent blood flow from neurohypophysis to adenohypophysis. Broken arrows represent the blood flow to and from the neural lobe. (Modified from Green.16)

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1028

lated to terrestrial habitat. The existence of the neurohypophysis and its hormones in fish in the absence of an evident capacity for renal response forces us to search for other functions.

The anatomy of the neurohypophysis in most fish differs strikingly from that in mammals (fig. 1). The neurosecretory nerve endings are either within the tissue of the adenohypophysis or closely applied to the capillaries that lead into the adenohypophysis. These neurons seem to be situated so that their secretions must reach the adenohypophysis in high concentrations. The lack of a neural lobe as such in aquatic vertebrates has been interpreted as indicating that the neurohypophysis in such forms functions as an organ of local secretion acting upon the adenohypophysis rather than as an organ for the systemic dissemination of hormones. Neurosecretory endings also occur in lower vertebrates in positions indicating that secretion occurs into the cerebral ventricles and possibly other parts of the brain itself.

The tetrapod neurohypophysis differs from that of fish in the development of a neural lobe (fig. 1). This structure appears, in a primitive form, in certain lungfish and is well developed in anuran amphibians and amniotes. The neural lobe is the caudal end of the neurohypophysis and it is characterized principally by its vascular relationships. Blood traversing its capillary bed does not pass to the adenohypophysis but into the systemic circulation. The neural lobe appears, therefore, well suited to function as an endocrine organ for the storage and general dissemination of neurosecretory hormones.

Local Actions of Neurohypophysial Hormones

The rostral portion of the neurohypophysis retains, even in mammals, an intimate relationship to the vascular supply of the adenohypophysis. Most, if not all, of the blood
reaching the adenohypophysis passes first through the capillaries of this portion of the neurohypophysis, the median eminence, before being carried to the adenohypophysis by portal vessels. This certainly suggests that neurohypophyseal secretions retain an action upon the adenohypophysis, and the physiologic evidence supporting this is growing rapidly.

Injection of huge doses of vasopressin intravenously causes increased adrenocortical secretion, possibly due to stimulation of ACTH release from the pituitary gland and from storage in other tissues and to a direct action upon the adrenal glands. Relatively small doses of arginine vasopressin instilled into the cavity of the third cerebral ventricle, however, appear to cause elevation of the rate of ACTH secretion in conscious dogs. Equimolar doses of 3 closely related peptides, oxytocin, arginine vasotocin (3-isoleucine, 8-arginine vasopressin) and oxypressin (8-leucine vasopressin), do not cause ACTH release when injected into the third ventricle. It is reasonable to believe that arginine vasopressin can diffuse from the third ventricle into the hypophyseal portal system and is carried to the adenohypophysis in relatively high concentration, causing ACTH release. Less direct action by way of other cerebral structures cannot, however, be excluded.

That arginine vasopressin in low doses can cause ACTH release when applied close to the adenohypophysis is far from proof that it is the neuroendocrine agent in hypophysial portal blood that causes ACTH secretion. Guillemin and others have obtained preparations from neurohypophyseal extracts in which vasopressor activity is considerably reduced relative to ACTH-releasing ability. It appears quite possible that releasing factors exist that are closely related to vasopressin or are derivatives of vasopressin. Whether they are physiologic mediators of secretory stimuli from the hypothalamus to the adenohypophysis remains to be demonstrated. This suggests, however, that neurosecretion of vasopressin-like peptides into the portal circulation may be an important physiologic link in the control of one adenohypophysial function by higher nerve centers.

A growing body of evidence suggests that oxytocin may influence adenohypophysial secretion of gonadotropins. Systemic oxytocin in large doses delays mammary involution in nursing animals after removal of the litter, presumably by maintaining luteotropin (prolactin) secretion. Oxytocin can also induce pseudopregnancy in rats, indicating that it can initiate pituitary luteotropin secretion. Large intravenous doses of oxytocin or vasopressin are reported to increase gonadotropin excretion in rabbits. It is conceivable, therefore, that oxytocin or oxytocin-like neurosecretions provide a means of hypothalamic control over gonadotropin secretion by the adenohypophysis analogous to the possible action of vasopressin on the adenohypophysial secretion of ACTH.

The suggestion that vasopressin suppresses thirst is an interesting one, although the evidence for this action is not strong. This would represent an action on higher nervous centers, modifying subjective sensations and behavioral responses. Vasopressin and oxytocin administered systemically in large doses are capable of causing spawning behavior in some fish, even after hypophyseotomy and castration. This is clearly an action on the central nervous system although it may not be a direct one. That oxytocin may retain actions directly upon cerebral structures in mammals is suggested by the extraordinary observation of Brooks and Pickford that small doses of oxytocin injected into the carotid circulation of conscious dogs produce a marked and persistent elevation of renal sodium excretion. Similar doses administered systemically are without appreciable effect.

Nature of Neurohypophysial Hormones in Mammals

The architecture of neurohypophysial tissue of mammals differs from that of other vertebrates. It is also of interest to inquire into pharmacologic differences and similarities with the expectation that information obtained
may increase our understanding of neurohypophysial function in mammals.

Arginine vasopressin has been chemically identified in the neurohypophyses of man,\textsuperscript{34} cow,\textsuperscript{35} sheep,\textsuperscript{36} and horse.\textsuperscript{37} Although there is no chemical proof, there is sound pharmacologic evidence for the presence of arginine vasopressin in monkey, cat, dog, rabbit, rat, and camel,\textsuperscript{38} and preliminary pharmacologic evidence for its presence in the opossum.\textsuperscript{39} The single known exception to this rule among the mammals is the domestic pig.\textsuperscript{40} The neurohypophysis of this animal contains lysine vasopressin. Since hog pituitaries are an important commercial source of vasopressin solution, one rarely knows, when he cracks an ampule, whether he is about to administer arginine or lysine vasopressin or a mixture of the 2. Whether this distinction is only academic as far as human patients are concerned has not been established.

As the antidiuretic hormone secreted by the pig, lysine vasopressin appears to be at least as potent as arginine vasopressin in causing antidiuresis in this species.\textsuperscript{41} Arginine vasopressin is, however, many times more potent than lysine vasopressin in causing antidiuresis in the dog.\textsuperscript{42} The rat is less discriminating, but the antidiuresis which results from lysine vasopressin is considerably shorter than that which occurs after arginine vasopressin.\textsuperscript{43-45} The antidiuretic responses to the 2 vasopressins in man have not been compared.

**Neurohypophysial Hormones in Nonmammalian Vertebrates**

Neither lysine nor arginine vasopressin appears to be present in those nonmammalian vertebrates that have been adequately studied.\textsuperscript{46} Pharmacologic and chromatographic studies on the chicken neurohypophysis have led to the conclusion that there are at least 2 active principles.\textsuperscript{47-48} One appears to be oxytocin. The second has pharmacologic activities that clearly distinguish it from any known mammalian hormone (fig. 2). We were extremely fortunate to have received, from Dr. du Vigneaud’s group at Cornell Medical College, many structural analogues of neurohypophysial hormones for pharmacologic study.\textsuperscript{49} Among these analogues was one called \textquoteleft arginine vasotocin.	extquoteright This chemical hybrid was synthesized by Katsoyannis and du Vigneaud.\textsuperscript{50} It has the ring structure of oxytocin and the side-chain of arginine vasopressin. The pharmacologic properties of arginine vasotocin match quite precisely the properties of the second principle in chicken neurohypophysial extract. If this pharmacologic evidence can be substantiated chemically through the isolation and identification of arginine vasotocin in the chicken neurohypophysis, Drs. Katsoyannis and du Vigneaud are in the extraordinary position of having synthesized a polypeptide hormone without being aware of its existence in nature.\textsuperscript{51}

Arginine vasotocin appears to be present in a greater quantity than oxytocin in the chicken neurohypophysis.\textsuperscript{47} It is much more potent than oxytocin in causing antidiuresis.
uterine contraction, and oviposition in the hen. This would indicate that arginine vasotocin is the antidiuretic hormone of the chicken and, possibly, is also involved in the process of egg-laying. The function of oxytocin in the hen remains obscure.

Employing similar methods with neurohypophysial extracts from a reptile, we have also tentatively identified arginine vasotocin and oxytocin. We have also obtained pharmacologic evidence that neurohypophyses from 2 anuran amphibians, a frog and a toad, contain arginine vasotocin and a smaller quantity of oxytocin. 

Bony fish appear to have active principles in their neurohypophyses quite similar, if not identical, to the principles in amphibians. Although arginine vasotocin is present, its function in fish remains a mystery. No clear-cut influence on renal function or water metabolism has been described. Depletion of neurosecretory material in hypothalamic nuclei and neurohypophyses in fish exposed to extremely concentrated external environments affords but weak evidence for a specific role of such secretion in the defense of internal osmotic integrity.

The elasmobranch neurohypophysis contains a peptide with biologic activities quite distinct from those of any of the neurohypophysial hormones or analogues that we have studied. There is no evidence that would suggest a function for neurohypophysial secretion in elasmobranchs.

The cyclostomes represent living remnants of the jawless fish that were ancestors of all vertebrates. The neurohypophysis of the marine lamprey, a member of this group, contains a spectrum of pharmacologic activities indistinguishable from that of arginine vasotocin. There is little or no oxytocin present. Again we have no information concerning the function of this peptide in the lamprey.

The Search for Related Principles in Invertebrates

Since active polypeptide principles occur in all major vertebrate groups, it is of interest to look for related substances among the invertebrates. Tunicates are believed to be closely related to the ancestors of the vertebrates. The large solitary ascidians (fig. 3) contain a structure that has a certain superficial resemblance to the vertebrate pituitary. This is the neural complex, made up of the remains of the larval brain, called the cerebral ganglion, and a closely applied glandular structure, the subneural gland. This gland has a duct which communicates with the pharynx. The analogy of this association to that of the neural and glandular portions of the vertebrate hypophysis has tempted many to suggest homology of function. The presence of neurosecretory cells within the cerebral ganglion also lends support to this hypothesis. Neurohypophysial-like activities have been sought and found in extracts of the neural complex. More critical investigations, however, reveal that the same weak oxytocic activity can be found in any tissue of the ascidian and that milk ejection, pressor and antidiuretic activities are essentially non-existent. The oxytocic activity resists alkali and sodium thioglycolate, unlike any known neurohypophysial peptide. The pharmacologic similarities between the neural complex and the neurohypophysis appear too superficial to offer any support for the argument in favor of homology.

We have, therefore, no knowledge of the prevertebrate evolution of the neurohypophysis. Neurosecretory systems analogous to the hypothalamic-neurohypophysal system are widespread among invertebrates. There is fragmentary evidence that certain crustaceans have neurosecretory products that can cause water uptake by frogs. This might indicate that principles similar to neurohypophysial hormones may exist in invertebrates. It seems extraordinary that such could be found in the eyestalks of crustaceans which are believed to be very distantly removed from the line of evolution leading to the vertebrates. We are left, therefore, with the thought that neurosecretary hormones may, throughout evolution, have common chemical characteristics. The alleged hormone in crustaceans that has frog water-balance activity may be an...
example of convergent evolution of neurosecretory hormones. Neurosecretory products resembling vertebrate neurohypophysial hormones may have existed in the invertebrate ancestors of the vertebrates. However, their presence in surviving forms related to the early vertebrates remains to be demonstrated.

**Vertebrate Evolution and Neurohypophysial Peptides**

Active neurohypophysial peptide hormones are present in all the major vertebrate groups. The active principles differ, however, from group to group. They have been chemically identified only in a few mammals. We have studied a small number of nonmammalian species pharmacologically. Using this pharmacologic evidence, it might be possible to formulate a tentative scheme for the evolution of neurohypophysial peptides based on the assumption that if 2 species share a peptide their most recent common ancestor also possessed that peptide. On this basis a phylogenetic scheme for neurohypophysial peptides is set forth in figure 4.

The Agnatha or cyclostomes are the most primitive living vertebrates. These jawless fish have a very primitive neurohypophysis that appears to contain arginine vasotocin. Proximity of the neurohypophysis to the vascular supply of the adenohypophysis suggests that neurohypophysial secretion in this primitive form is carried directly to the adenohypophysis. It is plausible to postulate, therefore, that the vertebrate neurohypophysis first appeared as an organ for local secretion modulating adenohypophysial functions.
Sharks and rays represent a line of evolution of jawed fish divergent from that leading to the bony vertebrates. In this group, a neurohypophysial peptide is present that is pharmacologically unique. We have no information concerning its chemical nature or its physiologic function.

The pharmacologic evidence indicates that the bony fish have retained arginine vasotocin. In addition, oxytocin occurs in this group, perhaps having evolved from arginine vasotocin by the substitution of leucine for arginine in the penultimate position of the side-chain. We have but meager clues as to the physiologic functions of these peptides in bony fish. The architecture of their pituitaries suggests that they function as local mediators between the neural and glandular divisions of the hypophysis rather than as systemic hormones.

The amphibians appear to have inherited the same peptides that are present in bony fish. They have, however, added the neural lobe, apparently as a structure for the systemic dissemination of neurosecretory products. Here one now finds that arginine vasotocin has acquired striking effects on the water permeability of the skin, the bladder, and the distal renal tubule. These actions promote water uptake through the skin, water reabsorption from the bladder, and water conservation by the kidney. Antidiuresis appears first, therefore, in amphibians as 1 of 3 mechanisms available for the retention and absorption of water and the protection of osmotic integrity. Arginine vasotocin appears to be the antidiuretic hormone of amphibians and presumably contributes to their ability to be amphibious.

Reptiles and birds do not seem to have altered the neurohypophysial peptides inherited from the amphibians. They have retained the antidiuretic response to arginine vasotocin. This peptide is also active in causing contraction of the oviduct in reptiles and birds. The function of oxytocin in these forms remains obscure.

Mammals have abandoned arginine vasotocin. Arginine vasopressin has displaced it as

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\text{CyS-Tyr-Phe-Glu(NH}_2\text{-Asp(NH}_2\text{)-CyS-Pro-Lys-Gly(NH}_2) \quad \text{Lysine vasopressin}
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\text{CyS-Tyr-Phe-Glu(NH}_2\text{-Asp(NH}_2\text{-CyS-Pro-Arg-Gly(NH}_2) \quad \text{Arginine vasopressin}
\]

\[
\text{CyS-Tyr-Ileu-Glu(NH}_2\text{-Asp(NH}_2\text{-CyS-Pro-Leu-Gly(NH}_2) \quad \text{Oxytocin}
\]

\[
\text{CyS-Tyr-Ileu-Glu(NH}_2\text{-Asp(NH}_2\text{-CyS-Pro-Arg-Gly(NH}_2) \quad \text{Arginine vasotocin}
\]

\[
\text{ASCIDIANS - NO ACTIVITY}
\]

\[
\text{ARTHROPODS - ? ACTIVITY}
\]

Figure 4

Hypothetic diagram of the phylogenetic relationships among neurohypophysial peptides.
the antidiuretic hormone. This step represents but a single amino acid substitution in the third position of the ring where phenylalanine replaces isoleucine. The domestic pig is the single known exception. Here lysine replaces arginine as the penultimate amino acid of the side-chain. This seems to be an isolated mutation, perhaps similar to that which occurred in the elasmobranchs.

Following its appearance in the bony fish, oxytocin persisted throughout subsequent evolution, its structure apparently inviolate. Its only established physiologic functions are related to lactation and, possibly, to mammalian parturition. Lactation is, of course, exclusively mammalian. Oxytocin does not appear to be directly concerned with egg-laying in birds or other nonmammalian vertebrates. It appears, therefore, that oxytocin evolved in the fish millions of years before the oxytocic or milk-ejection response.

F. L. Hisaw recently restated his generalization that, "It is not hormones which have evolved but the uses to which they are put." Although the primitive uses of oxytocin in nonmammalian vertebrates are unknown, new uses certainly evolved in the mammals. Arginine vasotocin is also present in fish, but antidiuresis first appeared in amphibians or their lungfish ancestors. This is another example of an ancient molecule adapted to a new use.

Hisaw also emphasized that his rule, like any good rule, does have exceptions. Antidiuretic hormones appear to offer an example of hormones that did undergo evolution while the "uses to which they are put" did not. The identity of the antidiuretic hormone changed twice during evolution, once from arginine vasotocin to arginine vasopressin and, in the pig, again to lysine vasopressin. The mechanism of antidiuretic action and its physiologic function, however, remained unchanged.

Neurohypophysial peptides evolved, therefore, long before their known peripheral actions. The primitive neurohypophysis appears to be an organ for local secretion into the blood supply of the adenohypophysis. This observation suggests that the neurohypophysis originated as a neurosecretory link between the brain and the adenohypophysis. A portion of the mammalian neurohypophysis retains a primitive anatomic relationship to the adenohypophysial circulation. We must seriously entertain the hypothesis that the mammalian neurohypophysis also retains a primitive physiologic function as a source of chemical mediators of hypothalamic regulation of adenohypophysial activities.

The term "antidiuretic hormone" is an imprecise one, necessitating a parenthetic identification. If reference is made to arginine (or lysine) vasopressin, the amino acid should be included in the name in order to define the hormone's chemical identity. Antidiuretic hormones appeared, however, far earlier in vertebrate phylogeny than did the antidiuretic response, and not as vasopressin. We would be wise, therefore, if we continue to use the term "antidiuretic hormone" or "ADH," to recognize that in the use of the adjective "antidiuretic" we may be prejudicing ourselves by emphasizing a recently acquired use to which these hormones have been put and discouraging our curiosity about other more primitive functions of possible physiologic importance.

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Circulation, Volume XXI, May 1960
ANTIDIURETIC HORMONES

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SAWYER, MUNSICK, VAN DYKE


**Down the Drain**

Elongated particles like threads or rods have a great tendency to associate and form structures. Such association is favored by the shape, the large surface, the poor balance of forces, and the friction resulting therefrom. Low motility and high viscosity will be characteristic of these particles and nature will invariably resort to this form wherever it wants to build structures. The "structure" of the cell is built of such particles and the really basic biological functions are linked to structure. Particles within this structure are mostly so intimately interlaced that they cannot be disentangled and isolated without profound damage. If we extract cells or tissues with water, a considerable part, the smaller half of the protein will be dissolved, but it will be globular proteins only which pass into solution while the basic structure will be left behind in the form of a semi-solid mass. Researchers did not know what to do with it and resolved the difficulty mostly by calling it "residue" and sending it down the sink. Unconsciously, research limited its attention therefore to the easily accessible globular proteins performing rather secondary functions around the basic structure and what we call today "protein chemistry" is, in its greatest part, only the chemistry of globular proteins.—A. Szent-Gyorgyi. *Nature of Life. A Study on Muscle.* New York, Academic Press, 1948, p. 10.