INTERNATIONAL ENCYCLOPEDIA OF PHARMACOLOGY AND THERAPEUTICS

Sponsored by the International Union of Pharmacology

Section 41 Volume I

Pharmacology of the Endocrine System and Related Drugs: The Neurohypophysis

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PHARMACOLOGY AND THERAPEUTICS

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VOLUME I

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1966

PERGAMON PRESS

OXFORD · LONDON · EDINBURGH · NEW YORK
TORONTO · SYDNEY · PARIS · BRAUNSCHWEIG

Pergamon Press Ltd., Headington Hill Hall, Oxford
4 & 5 Fitzroy Square, London W.1
Pergamon Press (Scotland) Ltd., 2 & 3 Teviot Place, Edinburgh 1
Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, New York 10523
Pergamon of Canada Ltd., 207 Queen's Quay West, Toronto 1
Pergamon Press (Aust.) Pty. Ltd., 19a Boundary Street, Rushcutters
Bay, N.S.W. 2011, Australia
Pergamon Press S.A.R.L., 24 rue des Écoles, Paris 5e
Vieweg & Sohn GmbH, Burgplatz 1, Braunschweig

First edition 1970

Library of Congress Catalog Card No. 69–18527

Printed in Hungary

08 013220 0

INTERNATIONAL ENCYCLOPEDIA OF
PHARMACOLOGY AND THERAPEUTICS

Sponsored by the International Union of Pharmacology (IUPHAR)
(Chairman: B. UVNÄS, Stockholm)

Executive Editor: G. PETERS, Lausanne

Section 41

PHARMACOLOGY OF THE ENDOCRINE SYSTEM AND RELATED DRUGS: THE NEUROHYPOPHYSIS

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VOLUME I

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INTRODUCTION

NOMENCLATURE OF NEUROHYPOPHYSIAL HORMONES AND RELATED PEPTIDES

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THE nomenclature of neurohypophysial hormones and their synthetic analogues, and indeed of other biologically active peptides, lacks uniformity. There are really two problems. Firstly the trivial or laboratory names of these peptides and secondly their generic chemical names. The mammalian hormones were called oxytocin and vasopressin long before their chemical constitution was known and these "laboratory names" were assigned by du Vigneaud and his colleagues to the nonapeptides whose structure they had determined. Similar trivial names were subsequently given to other naturally-occurring peptides in this series and to some of their synthetic analogues, e.g. vasotocin and oxypressin. The use of these trivial names is by now so common that they have been retained throughout this monograph. Objections have been raised from time to time against the use of the name vasopressin, since it describes a pharmacological rather than a physiological action of the hormone, and to ADH since the antidiuretic hormone of birds, for example, is arginine vasotocin. However, it was felt that provided it is understood that ADH refers to a mammalian antidiuretic principle (i. e. arginine or lysine vasopressin) contributors to this monograph should not be restricted in the use of either of these terms.

In numerous cases the full or abbreviated chemical name has to be used, and in these cases it has to be decided in what way an amino acid change in the molecule should be indicated. This problem has been considered by the IUPAC-IUB Commission of Biochemical Nomenclature who have issued tentative rules for naming amino acid and peptide derivatives (*Biochem. J.* **102,** 23–27 (1967) and **104,** 17–19 (1967)). It is proposed to follow these recommendations throughout this monograph. The rules state that in a polypeptide of trivial name, X, if the *q*th amino acid is replaced by another residue, the semitrivial name of the modified polypeptide is [q-new amino acid]-X and the abbreviated form is [Abc^{q}]X. For example [8-citrulline]-vasopressin or [Cit^{8}]-vasopressin. It follows from this that the naturally occurring vasopressins, for example, are written [8-arginine]-vasopressin and [8-lysine]-vasopressin.

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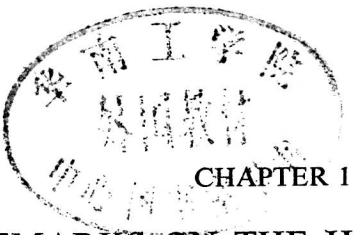

CHAPTER 1

REMARKS ON THE HISTORY OF NEUROHYPOPHYSIAL RESEARCH

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THE pituitary gland was well known to Galen as an anatomical entity and was so called because it was assumed to act as a filter or trap for the slime or *pituita*—the waste material excreted by the brain—which was then supposed to be conducted from the gland to the nasopharynx. This ancient concept of the pituitary as a gland of external secretion survived well into the eighteenth century (see Rolleston, 1936), but the celebrated author of the *Cerebri Anatome*, Thomas Willis (1664), seems to have rejected it since he wrote: "The ramification of the carotids into a reticulated plexus shows ... that the blood ... before it is led into the cerebrum takes some part of the superfluous serum of the pituitary gland and instils another part into the various shoots to be led back towards the heart." This explanation—as Harvey Cushing (1932) has pointed out—bears a curious resemblance to the concept of internal secretion, even though it was based on a factual misconception.

Recognition that the glandula pituitaria consists of two parts seems to have arisen at about the same period. For example, Thomas Willis seems to distinguish two lobes (but note that no division is shown in the plates of his *Cerebri Anatome)*, and is followed in this view by Vieussens (1684) and Winslow (1723). The Venetian anatomist Santorini (1724), however, appears to have been the first who recognized that the anterior part of the gland was not a continuation of the infundibulum. By 1766 the distinction between pars anterior and pars posterior is clearly made by A. von Haller in his *Elementa Physiologiae Corporis Humani* (1766), or to quote William Cullen's (1779) translation of the same author's *Primae Lineae Physiologiae* (1767): "This (the pituitary glandule) is compressed

on both sides, simple, of uncertain structure; in the anterior part almost round, and of reddish colour; the posterior part less, cinereous, broad transversely, covered with the pia mater of the brain: it lies upon the proper impression of the sella turcica, and seems to be a kind of appendix to the brain."

The nineteenth century saw very little progress in research on the neurohypophysis (as it came to be called after Soemmering in 1778 had introduced the term hypophysis as an alternative to "pituitary gland") probably because, when studied with ordinary methods of fixation and staining, the posterior lobe has a rather nondescript appearance. As late as 1889, for example, A. Macalister described the pituitary as "probably the rudiments of an archaic sense organ". The first and decisive advance—foreshadowing recognition of the physiological significance of the neurohypophysis—was made by Oliver and Schäfer (1895) who showed that the intravenous injection of an extract of the whole pituitary produced a rise in blood pressure. Shortly afterwards Howell (1898) proved that the pressor principle stemmed from the posterior lobe. Since then progress has been rapid and the directions it has taken will, for convenience of description, be described under the following headings: (1) morphology of the hypothalamo-neurohypophysial system; (2) chemistry of the active principles; (3) pharmacological action and biological function of the neurohypophysial hormones; (4) clinical use of neurohypophysial hormones.

MORPHOLOGICAL ASPECTS

Bundles of non-myelinated nerve-fibers entering the posterior lobe through the pituitary stalk were first described by Ramon y Cajal (1894) in two-day-old mice and later by Tello in 1912. Further studies showed that most of these fibers were derived from the supraoptic and paraventricular nuclei in higher vertebrates (Pines, 1925a, b; Greving, 1926) and the preoptic nuclei of lower vertebrates (Meyer, 1935).

Besides nerve fibers and their endings, the neurohypophysis contains glial elements (Retzius, 1894; Berkeley, 1894). Bucy in 1930 reported that these glial cells differed from the astrocytes and oligodendroglia of the adjacent brain and introduced the term pituicyte. Romeis (1940), who adopted this name, distinguished four types of pituicytes in man, one of which he compared with glandular cells and called adenopituicytes. He followed Bucy in the belief that these modified glial cells secreted the neurohypophysial hormones, a view which seemed to be supported by the observations of Gersh (1939) who claimed that osmiophilic granules accumulated in the pituicytes of rats deprived of water. However,

Gersh's findings could not be confirmed by Hickey, Hare and Hare (1941), or de Robertis and Primavesi (1942). Selye and Hall (1943) and Chambers (1945) described mitotic changes in the pituicytes of dehydrated rats, but it has been pointed out by Green (1947) that mitosis might have been due to unspecific stress. The functional significance of the pituicytes is thus obscure, but it should perhaps be noted that it has been stressed in a recent electron microscopic investigation (Lederis, 1965) that their processes often extend to the immediate vicinity of blood vessels, apparently ending near or in the perivascular space.

Another morphological feature of the posterior lobe and the neural stalk, described as early as 1908 by Herring, was the basophilic colloid masses or droplets which subsequently became known as "Herring bodies". Herring himself regarded them as cells derived from the adenohypophysis and his view was supported by Cushing and Goetsch (1910), but was contested by Carlson and Martin (1911) and Maurer and Lewis (1922). Some of the early investigators (e.g. Cushing and Goetsch, 1910; Collin, 1928) assumed also that the colloid droplets might represent the hormones of the neurohypophysis and this view became acceptable as concepts about the function of the neurohypophysial neurones began to change. Beginning with a description of hypothalamic neurones in a bony fish, *Phoxinus laevis*, in 1928 and continuing with investigations in other vertebrates including man, E. Scharrer developed the concept of neurosecretion, that is of nerve cells which, like other neurones in the central nervous system, show Nissl substance and neurofibrils but which, in addition, contain secretory material (for reference see Scharrer and Scharrer, 1954). Between the years 1930 to 1935 Scharrer then developed the concept that it was these neurones which had an endocrine function. This view was shared by Roussy and Mossinger (1934) but did at that time not gain general acceptance (see e.g. Ranson and Magoun 1939). The situation changed dramatically when Bargmann (1949) applied Gomori's (1941) staining method with chrome-hematoxylin-phloxine to the hypothalamo-neurohypophysial system. By this technique secretory neurones stain selectively and can be traced from the perikaryon in a hypothalamic nucleus to the nerve-ending in the posterior lobe, and it is seen that the Herring bodies are probably nothing but swellings in the course of individual nerve fibers. Bargmann assumed from the very first that the neurosecretory material containing the neurohypophysial hormones was formed in the nuclear cells and was transported to the posterior lobe from which the hormones are released. He and his co-workers (Ortmann, 1951; Hild and Zetler, 1953a, b) then showed in the subsequent years that the amounts of Gomori-positive material and antidi-

uretic activity in the hypothalamo-neurohypophysial complex are correlated and that, when the pituitary stalk is severed, neurosecretory material accumulates rostral to the cut (Hild, 1951; Hild and Zetler, 1953a). The new concept of the function of the hypothalamo-neurohypophysial system was summarized in a joint publication by Bargmann and Scharrer (1951) in which they postulated that "the pars nervosa of the vertebrate hypophysis stores but does not produce the stainable material which it contains. This material originates in the neurosecretory cells of the nuclei supraopticus and paraventricularis in the higher vertebrates and the homologous nucleus preopticus in the lower vertebrates; it passes to the pars nervosa by way of the hypothalamo-hypophysial tracts."

Further selective staining methods, useful for the light microscopical study of the hypothalamo-neurohypophysial complex and other neurosecretory systems, were the aldehyde-fuchsin method elaborated by Gabe (1953), the performic acid-Alcian blue method introduced by Adams and Sloper (1956), and the pseudoisocyanine reaction developed by Sterba (1961, 1964).

Numerous investigators have by now studied the hypothalamo-neurohypophysial system with the electron microscope and have shown that its ultrastructure is similar from the elasmobranch fishes (see Knowles, 1965) to man (Lederis, 1965). Two kinds of subcellular granules are recognized. One ranging in diameter up to 120 nm, called the "elementary granules" by Bargmann and Knoop (1957), was suspected from the start to contain the neurohypophysial hormones. Another family of smaller granules (about 60 nm, was originally called "synaptic", but this interpretation which was only based on morphological features has been contested (see Holmes and Knowles, 1960; Knowles, 1963; Lederis, 1963, 1965).

That the "elementary granules" contain vasopressin and oxytocin (and in the case of a teleost fish vasotocin, Lederis, 1962) has been verified by isolation of these subcellular entities by differential centrifugation and estimation of the hormone content of the fractions (Schiebler, 1952; Pardoe and Weatherall, 1955; Lederis and Heller, 1960; Heller and Lederis, 1962; Weinstein, Malamed and Sachs, 1961; LaBella, Beaulier and Reiffenstein, 1962; Barer, Heller and Lederis, 1963). It has also been shown by Ginsburg and Ireland (1966) that the distribution of neurophysin, the protein carrier of the active peptides in subcellular fractions, is essentially similar to that of the hormones.

CHEMICAL ASPECTS

Dudley (1919, 1923) found that the pressor and the oxytocic activities of posterior lobe extracts could be separated by extraction with butanol and he concluded in his paper of 1923 that "the uterine stimulant and pressor principles are two distinct chemical substances". In collaboration with H. H. Dale (Dale and Dudley, 1921) he studied the effect of proteolytic enzymes on these active principles, the results laying the foundation for the recognition that they are polypeptides. Further progress in the purification of the active principles was made when Kamm *et al.* (1928) and Stehle and Fraser (1935) introduced methods of separation by fractional precipitation from organic solvents. The procedure used by Kamm and his colleagues yielded a pressor fraction containing 160 units/mg and an oxytocic fraction of 300 units/mg. Kamm *et al.* estimated that each fraction was contaminated with 2–5% of the other hormone, but since it is now known (Munsick, Sawyer and van Dyke, 1960) that oxytocin has some "intrinsic" pressor-antidiuretic and vasopressin some oxytocic activity, it seems likely that the separation was complete though the products still contained some inert material. However, Kamm also prepared an oxytocic extract containing 500 units/mg, i.e. a preparation with the same potency as that of synthetic oxytocin.

Du Vigneaud and his colleagues (du Vigneaud, 1954/55) applied the technique of counter-current distribution to the isolation of oxytocin and vasopressin from fractions prepared according to Kamm *et al.* More recently the neurohypophysial hormones have been prepared by methods employing partly electrophoresis on a cellulose column (Porath, 1957), partly partition chromatography (Condliffe, 1955) and partly ion-exchange chromatography (Acher, Light and du Vigneaud, 1958). A method utilizing the specific binding of the hormones to neurophysin has also been successfully used for the isolation of the hormones of the ox and the pig (Acher, Light and du Vigneaud, 1958) and of other mammals including men. The use of non-homologous neurophysin for the preparation of non-mammalian hormones may, however, be hazardous since apparently vasopressin may be introduced into neurohypophysial extracts of species in which it normally does not occur (Munsick, 1964).

The structure of oxytocin was simultaneously and independently determined by du Vigneaud *et al.* (1953) and by Tuppy and Michl (1953). The same year saw the establishment of the structure of the vasopressin in ox neurohypophysial extracts (arginine vasopressin) by du Vigneaud, Lawler and Popenoe (1953). Popenoe, Lawler and du Vigneaud (1952) had already found previously that the vasopressin of the pig contained

lysine instead of arginine, thus furnishing the first example of the polymorphism of a neurohypophysial hormone (see Heller and Spickett, 1966). The synthesis of oxytocin followed in 1953–4 (du Vigneaud, Lawler and Popenoe, 1953), and that of arginine vasopressin in 1954 (du Vigneaud, Gish and Katsoyannis, 1954).

Comparisons of potency ratios obtained by bioassay techniques, of non-mammalian neurohypophysial extracts with the same ratios of mammalian gland extracts showed early (Heller, 1941a, b; Lazo-Wasem and Weisel, 1952) that non-mammalian pituitaries contained active principles related to but different from oxytocin and vasopressin. This technique of pharmacological characterization, in the refinement of which W. H. Sawyer and H. B. van Dyke were especially prominent, was substantially aided by the introduction of a simple paper chromatographic method for the separation and partial purification of very small quantities of neurohypophysial peptides (Heller and Lederis, 1958). Establishment of pharmacological spectra of activity, usually by a combination of chromatographic and bioassay procedures, led not only to the discovery of several new neurohypophysial hormones, but also—from the comparison with the profiles of synthetic analogues—to the tentative identification of several new hormones (see Chapter 3).

The discovery of these new neurohypophysial principles was much aided by the synthesis and pharmacological characterization of an extensive series of oxytocin analogues by a number of distinguished chemists amongst whom the names of Boissonnas, du Vigneaud, Rudinger and Šorm are particularly prominent (see Chapter 4). Their researches had the additional important result that structure–action relationships of this family of biologically active peptides are by now better explored than those of any other group of protein hormones (see Chapter 4).

PHARMACOLOGICAL ACTION AND BIOLOGICAL FUNCTION

It was apparent from the earliest work with mammalian posterior pituitary extracts that they exerted a variety of actions. As already mentioned, Oliver and Schäfer (1895) discovered the blood pressure raising effect in mammals. Dale in 1906 and 1909 showed that they contracted the uterus, and Ott and Scott in 1910 were the first to report the milk ejection effect of posterior pituitary extract. Farini and von der Velden, both in 1913, demonstrated their antidiuretic effect in man, Paton and Watson (1912) described the blood pressure-lowering action of undifferentiated neurohypophysial extracts in birds, and Brunn (1921) showed that mammalian posterior pituitary extracts injected into frogs produced

water retention—the so-called "Brunn" or "water balance" (Heller, 1941b) effect.

These investigations were subsequently extended—mainly by Herring (1913), Hogben and de Beer (1925) and Heller (1941a, b, 1942)—to extracts of non-mammalian neurohypophysial tissue and it could be shown that, qualitatively speaking, all vertebrate classes contain active principles which have the same biological effects as extracts of mammalian posterior lobes. Much work has also been expended—and is still being expended—on the problem of the physiological significance of these effects. Since administration of posterior pituitary extracts (or snuff) to patients suffering from diabetes insipidus reduced their excessive urine output to normal proportions, it was evident from the start that the pressor-antidiuretic principle (vasopressin) was normally involved in the regulation of water metabolism. Verney (1926), in experiments on the isolated head-kidney preparation, showed subsequently that the antidiuretic hormone had a renal site of action, and the same author (Verney, 1947) demonstrated that a small increase in plasma osmotic pressure led to a release of vasopressin from the neurohypophysis. These investigations led to the concept (see Chapter 8a) that water reabsorption by the mammalian renal tubule is under the control of the antidiuretic hormone and that this control is released when the plasma osmotic pressure falls as a result of fluid ingestion, the blood dilution leading to an inhibition of vasopressin secretion accompanied by the quick disappearance of the circulating hormone whose very short half-life has been demonstrated by Ginsburg and Heller (1953) and others (see Chapter 11).

The vascular action of vasopressin has, until recently, been regarded as an experimental artefact, mainly because antidiuretic effects can be produced without a demonstrable change in glomerular filtration rate. However, the suggestion has recently been made that effects of the hormone on the blood vessels of the renal medulla may be concerned in the physiological antidiuretic mechanism (Kramer, Thurau and Deetjén, 1960; Lilienfield, Maganzini and Bauer, 1961).

The demonstration that the injection of neurohypophysial extracts caused milk ejection in various species including man (Schäfer, 1913) suggested the possibility that the posterior pituitary plays a physiological role in the process of suckling. The first clear support for this concept derived probably from the work of Gaines (1915) who concluded that milk ejection was caused by a direct effect of posterior pituitary extract on muscular elements in the mammary gland. Ely and Petersen (1941) showed subsequently in cows that oxytocin was more effective than vasopressin in stimulating milk-ejection and postulated that the former hormone