Neuroendocrinology: a short historical review

Roger Guillemin
The Salk Institute for Biological Studies, La Jolla, California

Address for correspondence: Roger Guillemin, Ph.D., M.D., The Salk Institute for Biological studies, 10010 N. Torrey Pines Road, La Jolla, California 92037. guillemin@salk.edu

A short historical review from the early days of neuroendocrinology is presented recognizing hormones secreted by the pituitary gland; control of its functions by nuclei of the hypothalamus through the release of unknown substances in special capillary vessels; characterization of these releasing factors as peptides; studies of their mode of action; their use in clinical medicine; new and still ongoing demonstration of their ubiquity though not random in the brain and peripheral organs; and recent implications in control of behavior in animals and humans.

Keywords: neurosecretion; peptides; somatostatin; hypothalamus

To the best of my knowledge, the word “Neuro-Endocrinologie” (in French, with the hyphen) appeared for the first time in 1946 on the cover and as the title of an enormous treatise by Roussy and Mosinger. What I will recount here will be the successive observations, discoveries, and their interpretations that led to “neuroendocrinology” as we know it today as such and in its current extraordinary conceptual expansion.

In the 1800s, Volta and Galvani produced contractions of the muscles of the legs of dissected frogs by applying an electric current to the nerves of the spinal cord. Here, it was established that the nervous system functions as an electric machine.

However, in the 1860s, Claude Bernard showed that the chemicals in the poison “curare” will inhibit the effects of the electrical stimulation, thus concluding that some substance, other than the electrical changes of the potential involved when applying electrical current to the nerve, must be involved in the ultimate result of the electrical stimulation of the nerves. Thus, the concept of a neuromuscular junction was established.

In 1886, the neurologist Pierre Marie described and named “acromegaly,” which he considered, possibly, to be some sort of rheumatism. The pituitary gland as such was not even mentioned in his text, except for a note quoting a colleague practitioner who had observed an enlarged “mass in the sella turcica” during the autopsy of one acromegalic patient. But in those times, nothing was known about any function(s) of the pituitary gland.

In 1905, in one of the Croonian Lectures, Starling and Bayliss reported the existence in duodenal extracts of a substance, which they purified and named secretin, which will stimulate the secretion of gastric acid when injected intravenously. Starling introduced the word hormone, short for chemical mediator.

In 1904, the neurosurgeon Harvey Cushing performed the first hypophysectomy in a patient with a pituitary tumor compressing the optic nerve and, in 1912, published his book, The Pituitary Body and its Disorders.

In 1921, in one of the most astute experiments of classic Bernardian physiology, Otto Loewi, in Graz, Austria, showed that while electrical stimulation of the vagus nerve of an isolated heart will slow down or inhibit the contraction of that heart, the perfusion of another heart with saline irrigating the first heart will also produce similar changes of the heart contractions, thus implying that some substance is released at the contacts of the vagus nerve and the myocardial muscular fibers that can be transferred to another heart and produce the same effects without electrical stimulation of that heart. Loewi proposed the existence of some Vagustof involved and, 2 years later in 1923, Sir Henri Dale, in
London, showed the molecule in question to be acetylcholine, which he had been studying and had characterized in extracts of ergot. It was concluded that the stimulation of nerves releases some substance at the junction of the nerve and its target muscle.

Starting in the 1920s, several groups in France and the United States began to recognize that hypophysectomy in laboratory animals arrests body growth and inhibits functions of the newly recognized endocrine glands (thyroid, testes, ovaries, and adrenals). In the group around Herbert McLean Evans in Berkeley, the early purification, then isolation and molecular structure of six different hormones from the anterior lobe (GH, TSH, LH, FSH, ACTH, and PRL), was established. In 1952, Vincent du Vigneaud, and his group in New York, isolated, characterized, and synthesized two hormones—oxytocin and vasopressin—isolated from extracts of the posterior lobe of the pituitary. All these molecules of pituitary origin are peptides or proteins of various lengths (from 9 to over 100 residues).

In 1939, one of the most important events in the history of neuroendocrinology took place: a meeting in New York of the Association for Research in Nervous and Mental Diseases that revolved around the theme, “The Hypothalamus and Central Levels of Autonomic Function,” which was followed by the publication of its proceedings under the title *The Hypothalamus*. It was at this meeting that the Scharrers (Ernst and Berta) proposed the concept of neurosecretion by presenting histological images of neurons of the hypothalamus of a series of vertebrates and their equivalent in invertebrates, which are best explained by the concept of these neurons secreting substances, most likely of proteinic–peptidic nature.

And, in 1955, a book by Geoffrey W. Harris, *Neural Control of the Pituitary Gland*, appeared, which presented the results of Harris’ research showing that highly localized electrolytic lesions in the hypothalamus (rabbit, ferret) will specifically inhibit the secretion of one or another hormone of the anterior pituitary lobe, the electrical stimulation of the same locations leading to secretion of the same pituitary hormones. And since it had been established by that time that the anterior lobe—in contradistinction with the posterior lobe—does not receive nerve fibers of hypothalamic origin, the recently described capillaries in the pituitary stalk, extending from the median eminence to adenohypophyseal tissue were thought to be the conduit for some substances of hypothalamic origin that would act as necessary controllers of the secretions of pituitary hormones.

Jacques Benoit and Ivan Assenmacher, in France, were working with birds (primarily ducks), where the anatomy is somewhat different and had arrived at the same conclusion at about the same time. And Bernardo Houssay, in Buenos Aires, working with frogs and other animals, showed that the flow of blood in these capillaries is indeed from the brain to the pituitary.

Meanwhile, following his earlier observations published in 1936, Hans Selye in Montreal was developing his concept of the organism response to stress, in what he called “the general adaptation syndrome,” with an acute pituitary–adrenal cortex response to exposure to stress (the “alarm reaction”) followed by a stage of adaptation but ending in a stage of exhaustion and death, should the animal be exposed to extended periods of stress. In addition, the acute response of the adrenal cortex was shown to be prevented by hypophysectomy, thus implying a corresponding acute secretion of ACTH, which, in turn, could be prevented by one of the hypothalamic lesions as shown by Harris and others. And at the same time, Selye kept asking “What is the nature of this hypothalamic trigger of the stress-induced release of ACTH?”

As a student of Selye, I was early on intrigued by this question. Following the demonstration that none of the classic neurotransmitters (acetylcholine, norepinephrine, serotonin, etc.) was the answer, I decided to search for whatever substance coming from the hypothalamic neurons (cf. the images and the neurosecretion concept of the Scharrers) would trigger that pituitary response to stress. With the methodology of those days (1950s), it was out of the question to collect microliters of hypothalamic–hypophysial portal blood and isolate and characterize in it some unknown substance—assumed to be a peptide, as were vasopressin and oxytocin—which obviously would be present in very minute quantities.

In my own laboratory, now at Baylor College of Medicine in Houston, Texas, I first demonstrated the unquestionable existence, in crude extracts of hypothalamic tissues, of such a substance that triggered the release of ACTH in/by *in vitro* tissue...
cultures of the anterior pituitary. This unknown substance was named CRF, for corticotrophin releasing factor, on the proposal of Murray Saffran in Montreal, who had also been working on this problem. It became rapidly obvious that that substance was present in each fragment of hypothalamic tissue (from sheep) that we were obtaining at slaughterhouses, in extremely small amounts, to be processed and characterized with the then-available methodology. Eventually, we collected several million fragments of sheep brains weighing over 50 tons, and, in 1969, we were able, with the group at Baylor, to isolate the first of these hypothalamic releasing factors, TRF (TRH) releasing thyrotropin. Because of difficulties and uncertainties with the bioassays for ACTH, we had shifted from studying the release of ACTH to that of TSH as I had designed a highly reliable bioassay for such a substance. We showed that TRF was indeed a peptide, composed of three amino acids, glutamic acid, histidine, and proline, and we established the molecular structure by mass spectrometry as pyroGlu-His-Pro-NH₂. A synthetic replicate prepared for us by chemist friends at Hoffmann-La Roche Pharmaceuticals in Switzerland—at that time we were not equipped to synthesize peptides—had full biological activity. In some species (human and bovine) TRF also stimulates the release of prolactin. That molecular structure was confirmed about a year later in porcine hypothalamic extracts by the group of Andrew Schally—who had been my first post-doc fellow at Baylor and with whom we had been searching for CRF—now on his own.

All along, we had also shown the existence of a gonadotropin (LH) releasing factor and, in 1972, it was the group of Schally who first reported the complete structure of porcine LRF (LHRH) as that of a decapeptide that turned out to be also that of LRF (LHRH) of ovine origin as we established a few months later.

The synthetic replicates of these two peptides now available in large quantities were shown to be highly active in humans, and we started designing and synthesizing many analogs of these native sequences with the aim of producing superanalog—specifically in view of the short half-life of the native molecules, as well as competition antagonists in view of the multiple clinical implications.

By that time (1970), we had moved to the Salk Institute in La Jolla, California and had established a well-equipped laboratory, including the new radioimmunoassays developed by Sol Berson and Rosalyn Yalow, an extensive program of peptide synthesis by the new solid phase method of Merrifield, and, in our freezers, large quantities of ovine hypothalamic fragments. We then decided to look for the growth hormone releasing factor, the existence of which had been formulated as early as 1960 by Reichlin. We went back to our in vitro pituitary, adding crude saline or acetic acid extract of hypothalamus, following the secretory release of growth hormone by a just-established method of radioimmunoassay for rat GH. To our surprise, the addition of the crude hypothalamic extract inhibited the release of growth hormone, rapidly and in an obvious linear dose response. That was so unexpected that we suspected some mistake in the experiment; however, the observation was confirmed repeatedly. There was no previous solid evidence for a negative control of growth hormone secretion by the hypothalamus. But the results were so striking and consistent that we decided to proceed, and, in a few weeks, Roger Burgus, the chemist of the group, had isolated a 14-residue peptide that he sequenced, Jean Rivier synthesized, and that I named somatostatin. Antibodies were generated for possible radioimmunoassay, histochecmistry, and the synthetic somatostatin was largely distributed in response to many requests. It was also tested in collaboration with Sam Yen, University of California, San Diego, in some acromegalic patients with dramatic results in lowering blood levels of growth hormone. It was also observed in these patients and in normal individuals that infusion of somatostatin would lower glycemia and decrease levels of plasma glucagon and insulin, an observation that had first been made by the group around Charlie Gale, in Seattle working with baboons, but which we had never made in our studies in the rat. With a very short plasma half-life as we had measured it, and the circulation time between hypothalamus and periphery, if somatostatin was physiologically involved in the control of glucagon and insulin secretion, it had to be locally generated. In addition, I suspected that it would be found in the vagus terminals in the pancreas in keeping with its original discovery and location in hypothalamic nuclei. To everybody’s surprise, immunohistochemistry by Maurice Dubois, INRA, Nouzilly, France and independently Rolf Luft and Tomas Hökfelt in Stockholm, showed the peptide to be located in the δ-cells...
of the pancreas. This discovery was followed by the demonstration of the presence of somatostatin in the duodenum, the stomach, the small intestine, in several locations of the brain cortex and hippocampus, and some amacrine cells of the retina, along with five different kinds of receptors also recognized in several types of tumors. In all these locations, somatostatin was shown to be inhibitory to whatever were the classic functions of these tissues—organs. These investigations are still in progress, including the recent reports by Reubi et al. of specific analogs of somatostatin labeled with radioactive markers to localize not only the primary, but also the minute metastases of various kinds of tumors (pancreas, gut, lung). See also the report by Córdoba-Chacón et al. in this issue.

We still had to characterize a growth hormone releasing factor (GRF, GHRH). At that time (mid-1970s), several groups in the United States, England, and Sweden were reporting about the presence in the brain of receptors for opiates, suggesting the existence of endogenous opioid ligands. Since it was known that injection of morphine in animals, as well as in patients, was a strong stimulator of GH release as measured by plasma levels, we asked whether these endogenous opioid ligands could be the postulated GRF. Using our large store of hypothalamic fragments and a simple in vitro bioassay—the rat myenteric plexus—in a few weeks we had isolated three peptides of 13, 14, and 31 amino acid residues, which I decided to name enkephalins, a name suggested earlier by Eric Simon in New York. Then, as we were starting their characterization, there appeared the paper by Hans Kosterlitz and John Hughes that reported their isolation and sequencing of two pentapeptides with opioid activity, which they named enkephalins. To our surprise, the five residues N-terminal of our endorphins were identical to met-enkephalin, all of which turned out to be fragments of the molecule named β-lipotropin isolated years earlier by C.H. Li. In the bioassays, enkephalins and endorphins did release growth hormone in vivo but not by direct exposure to pituitary tissue in vitro. Thus, they were not the growth hormone releasing factor we were looking for.

Eventually, GRF (GHRH) was isolated, from two rare pancreatic tumors from two acromegalic patients without pituitary tumors, as a 44-amino acid linear peptide in our laboratory and that of Wylie Vale who had, in 1981, finally isolated from our ovine hypothalamic fragments the long sought after CRF, the corticotropin releasing factor—a 41-residue linear peptide of which we had shown the existence in 1955 but had never succeeded in isolating.

Thus, this discovery closed the search started in 1955 for the postulated hypothalamic releasing factors for each pituitary hormone, a search that also revealed the unexpected inhibitor somatostatin and many other facts of what is now neuroendocrinology and as exemplified in this congress.

Indeed, and again, the unexpected: every single peptide originally named and considered to be a “neuropeptide” because originally found in the cell body of neurons, has now been located along with its mRNA in practically all tissues where it has been searched for (see below). Similarly, many “peripheral” peptides (e.g., angiotensin, cholecystokinin, ghrelin, glucagon, leptin, melanocortin, secretin, etc.) have now been located in neurons—and some astrocytes—from which they are released with or without one of the “classic” neurotransmitters (5-HT, acetylcholine, norepinephrine, serotonin, etc.). Single type or, more usually, multiple types of receptors mediate in various tissues the pertinent, local activities of each and all of these peptides—for example, the presence of CRH, CRH-BP–binding protein, CRH-R1, CRH-R2 proteins and the corresponding mRNAs demonstrated in fat cells (human SZ95 sebocytes) where CRH as an autocrine secretion promotes lipogenesis.

In addition, there is increasing evidence of the synthesis and secretion of peptides by neurons throughout the brain. Oxytocin and vasopressin have been shown in multiple brain locations other than the hypothalamus where they were originally recognized, and their availability and release is being correlated with social aggressiveness or attachment; these observations were first done in rodents and then were confirmed in other species including primates and, very lately, humans. Similarly, there is evidence of extensive distribution in the cortex, the amygdala, and the cerebellum of CRH receptor 1 and 2 mRNAs, where the deletion of one or the other leads to different behavioral aspects (aggressivity, passivity) of the animals (mice, rats).

Since so many of all these effects can be correlated with the local, ubiquitous, though not random, presence of these peptides, their autocrine
release in many locations, along with their originally described hormonal characteristics, therefore, the concept of hormone as originally defined by Starling in 1905 may be reconsidered in view of the current and expanding observations mentioned here regarding the ubiquity of distribution and effects of these peptides. Thus, “neuroendocrinology” has become “neuro-psycho-entero-immuno-oculo-dermato-endo-crinology.” The superb content of the extended program of this 2010 congress is the obvious proof of that revolutionary statement.

**Conflicts of interest**

The author declares no conflicts of interest.

**References**