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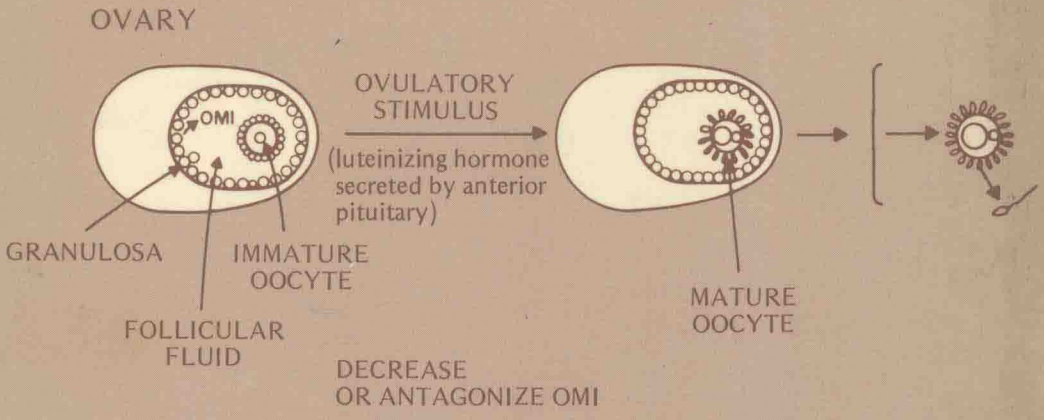
INTRAGONADAL REGULATION OF REPRODUCTION



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Intragonadal Regulation of Reproduction

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PREFACE

Advances in radioimmunoassay (Yalow and Berson, 1960, *J. Clin. Invest.* 39, 1157-1175) have led to substantial developments in knowledge in the field of endocrinology, particularly in reproductive endocrinology. Since 1965, many investigations have provided data on the hormonal events of puberty and menopause, adult testicular function and the course of the menstrual cycle, during which follicular development is followed by ovulation and the formation and functioning of the corpus luteum. In addition, biochemical investigations have led to an improved understanding of the hormonal basis of a large number of syndromes, such as infertility, amenorrhea, etc... and as a result, to improvements in therapy.

However, a number of physiological and physiopathological problems remain to be solved, which are not explicable from knowledge of the secretory characteristics of the gonadotrophins, prolactin and the sex steroids. Thus, there is a lack of understanding of why, in each menstrual cycle, about 500 follicles begin to develop under the influence of increased FSH secretion, while only one is ovulated, the remainder becoming atretic.

Likewise, why do all oocytes not re-enter meiosis at the time of the ovulatory LH peak? How can the sudden morphological and functional change in the granulosa cells after rupture of the follicle be explained? Finally, what are the causes of luteolysis, when gonadotrophin secretion does not change?

In the male, close relationships exist between the tubules and the interstitial tissue but their importance in spermatogenesis and in androgen secretion remains uncertain.

Such problems have begun to be explained since the discovery of steroids and polypeptides produced by the ovary and/or the testis, which are able to control locally the functional activities of the different gonadal compartments. They include substances which fulfil the definition of cybernins (Guillemin, ch. I). These cybernins,

produced by the ovary and the testis, modulate the gonadal response to stimuli released at a distance, such as the gonadotrophins, prolactin, etc... This book is devoted to a description of current knowledge of these substances produced by and active on the gonad: gonadal steroids, oocyte maturation inhibitor (OMI), the gonadotrophin binding inhibitors and stimulators, luteinization stimulators and inhibitors, the gonadocrinins, the epididymal maturation factors. Furthermore, inhibin is the subject of several chapters. It is firstly a hormone, produced by the gonad, and involved in the regulation of FSH secretion. A number of reasons exist for devoting part of this book to this hormone, the existence of which has been postulated for more than 50 years, but proved only in the last few. In the first place, it is a protein hormone like the currently known ovarian and testicular cybernins. It does not therefore share the steroid characteristics of the gonadal hormones. Furthermore, it is possible that it has local effects which allow it also to be considered as a cybernin. Finally, nothing is known of the structure and biochemical relationships which exist between the various protein cybernins and inhibin. It is possible that these substances differ from each other chemically and biologically. But it is also conceivable that the same substance has several biological properties. Finally, one can postulate the existence of a high molecular weight precursor, which, as a result of enzymatic cleavage, would release one or other cybernin.

The recent demonstration of intragonadal peptides which act locally and modulate the effects of hormones secreted at a distance opens up wide perspectives in our understanding of physiological and pathophysiological reproductive processes. In addition, these cybernins are potential biochemical weapons capable of interfering with fertility. This is the reason why we considered that the time had come to edit this book entitled: "Intragonadal regulation of reproduction".

April 1981

Paul Franchimont
Cornelia Channing

CONTENTS

CHAPTER I
ON THE WORD: CŸBERNIN

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The following note was written at the kind request of the Editors of this book. As will be obvious, it represents the early expressions of some still unfinished thought. Thus, nothing would please me more than to stimulate some discussion on the occasion of this short text. The fate of the word "cybernin" may be in its demise or in one avatar or another that I may not have envisioned. I am quite prepared for any of these possibilities.

When we started working on isolating the substances involved in the hypothalamic control of pituitary functions, the nomenclature proposed for them was that of releasing factors, hypothalamic releasing factors (Guillemin, 1978a). Later on, when three of those substances were isolated, characterized and reproduced by total synthesis, Schally proposed calling them hormones, releasing hormones (Schally, 1978; Schally *et al.*, 1968). The criterion for the new proposal that would differentiate releasing hormones from releasing factors was that of isolation and characterization of the primary entity involved in such-and-such a hypophysiotropic function which, while still uncharacterized, would have been called a releasing factor. Schally's proposal was followed by practically everybody and it is true that it is convenient to talk of "brain hormones". The question must be asked, however, whether the substances in question fit the definition of a hormone. The word hormone was originally introduced into the literature by Ernest Henry Starling on June 20, 1905, in the first of his Croonian Lectures delivered before the Royal College of Physicians in London on "The Chemical Correlation of

the Functions of the Body". Starling frames the thinking within which the definition of hormones will appear as follows (p. 339):

> The chemical adaptation or adaptations of the body, like those which are carried out through the intermediation of the central nervous system, can be divided into two main classes - 1) those which are involved in consequence of changes impressed upon the organism as a whole from without; and 2) those which, acting entirely within the body, serve to correlate the activities, in the widest sense of the term, of the different parts and organs of the body.

Then, after some discussion of the first category, he moves on to the second and says the following (p. 340):

> These chemical messengers, however, or hormones (from ormao, I excite, or arouse), as we might call them, have to be carried from the organ where they are produced to the organ which they affect, by means of the blood-stream, and the continually recurring physiological needs of the organism must determine their repeated production and circulation through the body.

Most of the substances which we call hormones to this day do meet these criteria; this is the case for the secretory products of all the endocrine glands. These circulate far and wide from their organ of production to the receptors of their target organs, where they somehow excite, stimulate or cause some positive effect in the cells of that tissue. Physiologically meaningful levels of these hormones can now be measured by all sorts of exquisitely sensitive methods in the peripheral blood, in blood samples anywhere in the body, with the added feature that there is always a demonstrable arterial/venous difference in the concentration of these substances when measured in the in-flow or out-flow blood to and from the organ known to be the source of the hormone in question.

In the case of the hypothalamic peptides involved in the control of pituitary functions, those that we know today (i.e. the tripeptide pGlu-His-Pro-NH_2 controlling the secretion of thyrotropin and prolactin; the decapeptide controlling the secretion of both gonadotropins; the tetradecapeptide somatostatin, known to inhibit the secretion of growth hormone), there is now reliable evidence that they can be demonstrated by bioassay or radioimmunoassay in the effluent blood from the hypothalamus when tapped in the portal vessels along the pituitary stalk (Porter *et al.*, 1977). There is also no doubt that there is a difference in the concentrations found in the hypothalamic portal blood when compared to peripheral blood (Chihara *et al.*, 1979; Gillioz *et al.*, 1979). This, however, is where the problems begin in calling these substances hormones. Reliable methodology shows that the peripheral levels of circulating thyro-

tropin releasing factor (TRF) or gonadotropin releasing factor (LRF) are so low as to be of no physiological significance. In the case of somatostatin, the matter is even more complex. First of all, somatostatin is universally an inhibitor (of one secretion or another), and thus can hardly be called by a name that etymologically implies "stimulation, excitation". But more importantly perhaps, it is now well recognized that somatostatin has a ubiquitous distribution (though not random) ranging from the central nervous system to multiple locations of the gastro-intestinal tract and the pancreas (Guillemin, 1978b). Immunoreactive and bioactive somatostatin, in fact forms of somatostatin of various molecular sizes, can be demonstrated to circulate in peripheral blood (jugular vein in laboratory animals, antecubital vein in man) (Benoit *et al.*, 1980), but in concentrations that appear to be far below what can be calculated to be binding or affinity constants. There is, however, excellent evidence that much larger concentrations of immunoreactive and bioactive somatostatin can be shown in more localized circulation such as, for instance, in the effluent vein of the pancreas, where we know that somatostatin is present in the delta-cells. There is also good evidence that those local plasma concentrations of local somatostatin can vary considerably as a function of physiological or experimental situations (absorption of meals, injection of various peptides, such as cholecystokinin, or endorphins, or drugs such as opiates, or arginine) (Schusdziarra *et al.*, 1980). If TRF, LRF and somatostatin are to be called hormones and considered as such, then it must be said that they do not qualify as the classical hormones.

But things are even more complex. We know now that immunoreactive, as well as bioactive, TRF, LRF and somatostatin are found within discrete neurones, either in the cell body or in peripheral endings from which they certainly have to be released for physiological purposes not well understood for the moment (review in Guillemin, 1978b). In such circumstances and setups, neither TRF, LRF nor somatostatin behaves as, or meets the criteria of, hormones. They seem to be involved in localized controls. It is probably also the case when trying to understand how pancreatic somatostatin could modify the secretion of insulin and glucagon by the nearby cells of the islet.

Because of their neuronal locations, TRF, LRF, somatostatin (and the same may also be true for other biologically-active peptides such as neurotensin, endorphins, enkephalins, VIP, etc) have been proposed as neurotransmitters, as are catecholamines or acetylcholine. But somatostatin is not a neurotransmitter when released by the delta-

cells of the endocrine pancreas to affect the glucagon secretion by a nearby alpha-cell, reaching either through gap junctions or extracellular space. Noradrenalin in and out of neurones is the neurotransmitter, while adrenalin is the hormonal form in and from the adrenal medulla, with the small amounts of noradrenalin found in the adrenal medulla leaving us in a quandary.

There are other recent complications. We have recently observed, as described in this volume (see chap. VIII) that follicular fluid contains a substance, peptidic in nature, that has been shown in various bioassays both *in vitro* and *in vivo* to have the full biological activity of the hypothalamic gonadotropin releasing factor to stimulate the secretion of LH and FSH. That substance is not chemically identical to the hypothalamic decapeptide releasing gonadotropins. There is no knowledge, at the time of writing of this note, as to whether this molecule, which for the ease of operation we have termed "gonadocrinin", reaches the peripheral circulation, thus possibly being active at the level of the pituitary. There is, however, increasing and solid evidence that the ovary contains high-affinity binding sites for the hypothalamic gonadotropin releasing factor, as judged from binding studies with labelled LRF or any of its active agonist or antagonist analogs (Hsueh and Erickson, 1979; Clayton *et al.*, 1980). There is also good evidence that, likely through these binding sites, the hypothalamic decapeptide or its superagonist analogs can profoundly modify the ovarian response to gonadotropins and also inhibit the secretion of estrogens (Hsueh and Erickson, 1979). It is difficult to relate the presence of these binding sites for LRF in the ovary to the peptide of hypothalamic origin since, as we have said above, the concentrations of circulating LRF are infinitesimal (lower than 10^{-12} M); thus the keen interest at the moment in our demonstration of the existence of gonadocrinin, an LRF-like peptide of ovarian origin which may be the true ligand for these ovarian receptors or binding sites. Obviously this LRF-like molecule (gonadocrinin) of ovarian origin, and when in the ovary, works neither as a hypophysiotropic releasing factor nor as a neurotransmitter.

It is obvious that the current terminology is wanting. Either we have to re-define what it is that we mean by hormone or some additional terminology has to be proposed.

The question is what to do with these molecules which have local effects that can range from angstroms to microns (gap junctions, extra-cellular spaces) in and from cells which are not neurones, to centimeters, when dealing with local either splanchnic or pituitary locations. Such substances do not fit in well with the definition of a

hormone. We have the word paracrine, as originally proposed by Feyrter (1938, 1946) to describe precisely the suspected secretory activities of what we now know to be the peptide-secreting cells of the gut. The etymology of the word is obvious and implies indeed a local or nearby use or function for what is being secreted. I personally think that the word paracrine is excellent and should be used often in relation to the problem which we are discussing here. But paracrine is an adjective, and to my knowledge Feyrter, in his difficult German, used it exclusively as such, referring to paracrine secretory cells and paracrine secretion. We could perhaps coin the word "parahormone" or "parhormone", but neither is euphonic or easy to pronounce in either French or English, or German for that matter. Several years ago I proposed the word cybernin, from the Greek "kurbenetes", meaning "pilot" or "rudder" of a boat, implying the local nature of the command or information involved. This is also the root of the well-known word cybernetics, even though I could never ascertain whether Norbert Wiener implied any localization (of information) when he decided to use the word (according to Littré, the word "cybernétique" was coined by Ampère to define "la partie politique qui s'occupe des moyens de gouverner"). I never pushed very hard for the implantation of the word cybernin, feeling that it was another word, without a clearly defined mission. The word, however, is being used by more and more people, thus appearing to fulfil a role; hence this short chapter here. So, how should we use the word? First of all, what is a cybernin? A cybernin is a polypeptide biosynthesized, processed and released by a cell or group of cells that represents (is) information that will affect the function of another cell or group of cells in the vicinity of the first cell or group of cells. Such a simple definition excludes all steroids, prostaglandins or molecules such as cyclic-AMP. Would beta-endorphin, ACTH, which certainly are hormones when secreted by the pituitary, be considered as cybernins when relating to their presence in hypothalamic neurones and when released either at nerve endings from these neurones – a statement which is only a proposal at the moment, albeit a logical one – or when released in the portal vessels of the pituitary from the median eminence and as measured in the down-flow blood along the pituitary stalk? The answer is yes – I would say that, in these circumstances, beta-endorphin and ACTH are seen, act, and should be considered, as cybernins. When we describe their action it will be referred to as a cybernin action rather than a hormonal action. Gonadocrinin in the ovary will work as a cybernin, but it would work as a hormone if it could be shown that it circulates in peri-

pheral blood and eventually reaches the pituitary and acts on pituittary receptors to modify pituitary function. Somatostatin will act as a cybernin when it modifies the secretion of insulin and glucagon in nearby pancreatic cells and originates from pancreatic delta-cells; it will also be acting as a cybernin when proceeding to the adenohypophysis, inhibiting the secretion of growth hormone, when originating from the hypothalamus. I would prefer to consider somatostatin as a cybernin rather than as a neurotransmitter if it can be shown that it is actually released at some axonal or dendritic ending and that it modifies the response of another neurone to any one of the classical neurotransmitters. In fact, the word cybernin may turn out to be the optimal noun for the adjective paracrine. The polypeptide growth factors recently proposed by Sporn and Todaro (Sporn and Todaro, 1980) as "autocrine" secretions in the ultimate of paracrine function could also be considered as cybernins,

The success of any nomenclature is based on need. The need for the use of the word cybernin is probably not compelling; only partially so. However, since the words hormone, neuromediator, mediator, modulator, are either too restrictive or too vague in their definition or implied use, maybe there will be some feeling of comfort in the use of the word cybernin. May I repeat that I wish this short text to be reflected upon only in the spirit of its opening statement.

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