Review Article

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MOLECULAR ENDOCRINOLOGY- A PERSONAL PERSPECTIVE

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Abstract: The philosophical underpinnings of Molecular Endocrinology have been examined. The major questions raised by molecular endocrinology and the progress achieved in answering these three major questions have been presented as a historical development of connected conceptual ideas in this area. The unsolved problems of this area of research have also been brought to notice. It has been shown that endocrinology is the most elegant example for the success of Reductionist philosophy in Biology.

Keywords: Hormone Action, Reductionist Biology, Endocrinology, cAMP, Receptors.

INTRODUCTION

Discovery of hormones and endocrine phenomena:

Experimental work in endocrinology is an excellent example for the reductionist view of biology. This view point, which is currently in fashion, asserts that the laws of physics and chemistry are sufficient to understand and to 'explain' biological phenomena. While this statement will not be discussed any further, it cannot be helped saying that this view point has dominated and influenced research and hence, available knowledge in endocrinology. In fact, the very process of discovery of hormones establishes firmly this philosophy of reductionist biology as far as endocrinology is concerned.

Physiologists and experimental anatomists have always tried to establish the cause and effect relationship between a given tissue and a biological phenomenon. Hence the same approach was taken up in classical endocrine research i.e. establishing the link between a suspected endocrine gland/tissue and a physiological process. Surgical extirpation and replacement therapy was the popular experimental approach adopted in this area. A tissue/gland is surgically removed and the putative chemical factor identified. The classical experiments of Berthold in 1849, where he had removed testes from chicken and then either replaced them back in the same site or put them in the animal's body cavity away from the original site, showed that the growth of comb and wattle was not affected when the chicken grew into adult birds. However the birds with testes removed but given no replacement did not develop the sex hormone dependent comb or wattle. This proved that a chemical substance from testes 'diffused' through blood and acted on tissues far removed from the site of synthesis. However it was only when Starling, in 1903 coined the term 'hormone' to a GI tract diffusible chemical that the field of endocrinology officially started off. Subsequently a similar approach was taken for discovering a number of hormones. This is based on the principle that a chemical is responsible for the physiological phenomenon. It will be obvious that scientists would try to purify the chemical. The endocrinologist would now administer the purified chemical into the surgically extirpated animals and demonstrate that all the parameters of body physiology which were lost upon surgery (extirpation) are now restored to homeostatic levels upon administration of putative chemical/hormone. This establishes that a chemical alone can cause the physiological phenomenon. Purification of hormonal substances, therefore, has been practiced for over six decades as a legitimate research activity [1]. Physico-chemical characterization of the purified hormone is the next obvious research activity. The physiological interpretation of endocrine phenomena implies that hormones are responsible for a number of biological phenomena and hence fluctuation in hormone levels in circulation would 'explain' for the absence or presence of a number of physiological phenomena. This would automatically raise the question as to what brings about the fluctuations in circulatory hormone levels. Progress in the basic area of Biochemistry and molecular biology enabled scientists to look into the molecular details of hormone biosynthesis, assembly, and secretion from their respective endocrine tissues of origin. This area has revealed very exciting details about the process of cell secretion. The regulation of secretion of a hormone has been investigated in fair detail at the histological, electron microscopic and molecular levels by appropriate techniques.

For more than four decades now, endocrinologists have recorded the various effects of

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administration of purified hormones into animals belonging to different phylogenic groups. A huge body of information exists [2-4]. The concept of receptors has brought to focus the problem of mechanism of hormone action. Understanding the structure and physiology is obviously the next concern of endocrinologists. Considerable progress has been registered in the membrane receptors as well as in the case of nuclear receptors. One of the spin offs from this area of study has been the bioassays based on reconstituted responsive systems where rDNA technology based receptors and a reporter gene like that of luciferase are used. A number of receptors have been cloned and expressed [5].

A recurrent theme in endocrine physiology is the question of relating molecular structure to function of a hormone [6-7]. The structural basis of hormonal activity has been elusive to find. In fact the discovery of orphan receptors without any known hormonal ligand to bind and reports of mutations in the receptor gene leading to constitutively activated state of a receptor has created a new endocrinology where there are no hormones!

Mechanism of action of hormones acting through membrane receptors:

Ever since the discovery of hormones, endocrinologists have documented thousands of effects produced by the administration of hormones into recipient animals. Hormones of different levels from crude extracts of glands to purified samples have been used for this purpose. Analysis of various effects brought out in late nineteen fifties, some general features of hormonal effects [8]. These are one, compared to the amount injected; the effects are highly amplified meaning thereby that hormonal effects are not stoichiometric but catalytic in nature. Two, some effects are clearly metabolic while others related to growth and development. Three, a certain degree of specificity exists as a given hormone affects only its target tissue physiology. Consequently hormones were arbitrarily classified into two classes- Metabolic Hormones and Growth & Developmental hormones. A possible mechanism of hormone action still eluded endocrinologists till late nineteen fifties. The specificity of hormone action led to the discovery of Receptors [9-11]. These receptors were supposed to be localized in the respective target tissues. The coincidental sub cellular location of receptors on plasma membrane in the case of metabolic hormones and intra cellular in the case of developmental hormones apparently validated the arbitrary classification. Exceptions like Growth hormone, Thyroid hormone etc were conveniently ignored.

Identification of Receptors:

The membrane receptors were identified through the use of radio labeled hormones hence forth called ligands. Labeling procedures were developed. The membrane receptor work has led to at least half a dozen Nobel Prizes (Sutherland, Rodbell, Gilman, Greengaurd, and Krebs to name a few) [12-13]. The discovery of adenosine-3'-5' cyclic monophosphate (cAMP) as a second messenger of hormone signaling is a major chapter in the field of hormone action. Sutherland and his associates made this epoch making discovery quite serendipitously [23]. This discovery also solved the age-old mystery of how hormone action could not be demonstrated in cell free preparations. The idea of second messenger was quickly confirmed. The mystery of how cAMP could mediate diverse actions of many hormones was clarified with the discovery cAMP dependent protein kinase and later other protein kinases. The laboratory of Carl Cori and Gerta Cori in Vanderbilt University where the whole problem of regulation of Glycogen metabolism was initially being investigated, through the brilliant investigators like those mentioned above, essentially brought the story to this level in a span of forty years.

The concept of second messenger was extended to situations other than hormone action and included most of the signal transduction pathways. Subsequently, other second messenger systems like IP3, Ca++, cGMP etc., were discovered. A system of protein kinases, phosphor protein phosphatase and inhibitors of phosphatase could 'explain' the mechanism of mediation of hormone action by cAMP. Philip Cohen of the University Of Dundee, Scotland made a major contribution.

One of the puzzles of hormone action is hormone inaction. Of the number of physiological situations where there is no tissue response to a hormonal signal (e.g. pre pubertal condition where gonadotropin levels in circulation is higher than in adult), the period immediately after the hormone injection when the intensity of response after the initial rise comes down to a stop is difficult to understand. The work of Gilman and Philip Cohen partially gave answers. Gilman's work using cytogenetic and biochemical methods led to a model describing the molecular events within the cell membrane when a hormone attaches to the receptor. A GTP-GDP exchange on the G-protein, the third component of the majority of membrane receptor systems was proposed as the mechanism of cessation of hormone action. Years before this, Rodbell had noticed the strange phenomenon of hormone coming off the receptor precisely at the time when GTP addition initiated hormone action (tissue response, in other words) was beginning. 'Gilman cycle' explained it in the form of GTP-GDP exchange. A ternary complex of receptor-G protein-adenylate cyclase proposed as

the molecular basis of hormonal action in terms of stimulated adenylate cyclase activity while GTPase activity of the G-protein would hydrolyze GTP to GDP and terminate thus hormone action. GTP and Hormone were supposed to induce each other's dissociation/binding. Philip Cohen's work on phosphorylase kinase also gave another focal point in the downstream processes of the post hormone-receptor interaction

The consensus picture of hormone action through G-protein linked membrane receptors is hormone binding to the signal discriminating unit of the receptor complex leads to activation of an effector system like adenylate cyclase or phospholipase C via interaction with a specific G-protein. A cascade of events involving protein kinases, appropriate substrates for kinases, and the respective phosphoprotein phosphatases would result in the specific physiological response. The diversity of hormonal effects was explained by the presence of different substrates for protein kinases in different tissues. An expanded concept of membrane receptors would include those cases in which the effector system could be ion channel or another enzyme system. It also includes systems where G-protein involvement is not clear. An example of the latter is cases where the receptor is also a Tyrosine kinase. Most of the G-protein interactive membrane receptors have been cloned and expressed. The chemistry of these receptors has been worked out [14]. Fragments of receptors have also been shown to be present in the target tissues. The physiological significance of this observation is not clear [15]. The second major receptor system whose mechanism of action is fully clear is the intracellular nuclear receptors. Steroid hormones, thyroid hormones and some others interact with nuclear receptors.

Mechanism of action of Steroid hormones-nuclear receptors:

Developmental hormones like estrogens, androgens etc essentially bring about growth and differentiation of their respective target tissues. When examined carefully, however, one would notice that the gross effect of steroid hormones actually exhibits a variety: These are one, decrease in cell number and hence target tissue atrophy (e.g. glucocorticoid action on thymus); two, no change in cell number (e.g. estrogen action on hepatic tissue); and three, increase in cell number in the target tissue including differentiation (e.g. estrogen action on hen oviduct). Translated into molecular terms, steroid hormone action results in differential gene expression. Hence transcriptional regulation of target tissue/cell gene expression is a unifying molecular paradigm of steroid hormone or any developmental hormone (like thyroid hormone on brain etc.) action. The most dramatic example of a developmental hormone is strangely thyroxine, whose metabolic effects in homoeothermic animals are a long standing paradigm in endocrinology. Thyroxine in fact has an equally major role in development, the classical examples being amphibian metamorphosis, brain development in mammals etc.

As in the case of metabolic hormones, use of radio labeled steroid/thyroxine led to the discovery of receptors. A spectrum of proteins with variation in affinity for a particular steroid hormone is involved in the binding, transport and response to that particular hormone. These include the serum binding and transport proteins, the cytoplasmic binding proteins in the target cells, the membrane binding proteins in certain cases (mediating the non-genomic actions of steroid hormones) and the nuclear binding and responsive receptors proteins of the target tissue. Although Elwood Jensen got a Nobel Prize in discovering the estrogen receptors in the cytoplasm of target tissue, most people believe that steroid receptors are in the nucleus.

The nuclear receptors have been purified by biochemical procedures as well as cloned and expressed by rDNA protocols. Structural analysis of these proteins has revealed that they have supra secondary structural motifs like Zn-finger, helix-loop-helix, Leucine zipper etc which appear to be characteristic of DNA binding proteins like transcription factors. In addition, following hormone administration, rates of target tissue macromolecular synthesis including that of protein, RNA and sometimes of DNA invariably are found to increase. A number of model systems like estrogen action on hen oviduct leading to egg formation, glucocorticoid action on thymus leading to its involution, androgen action on male accessory sex organs like prostate and seminal vesicle, estrogen action on liver leading to vitellogenesis in oviparous animals have been analyzed extensively and intensively. Molecular details of the interaction of the receptor with the hormone on one hand and DNA on the other hand have been worked out. Although the final detailed picture is not very clear, a conceptual account of steroid hormone action is possible. Thus the steroid hormone bound receptor interacts with specific cis-acting elements called enhancers/silencers to influence the basic transcriptional machinery of the target cell so that a specific gene transcription is regulated up (enhanced) or down (silenced). Two complications in steroid hormone action have eluded understanding. One, activation of receptor without the ligand (agonist) and two, the presence of orphan receptors. The latter were discovered by what is called reverse endocrinology approach. Consequent to the progress in the area of biochemistry of steroid receptors, molecular biologists noticed, in genomic and cDNA libraries, DNA clones which have sequence similarity to nuclear receptor gene sequence. They

could be translated in vitro into receptor like proteins. But natural agonists for this nuclear receptor like proteins are hard to identify. To add to further complication, a number of diet derived small molecular weight organic compounds (simple fatty acid derivatives) when administered into recipient animals could induce developmental effects, possibly through such orphan receptors. However in many cases, direct binding experiments failed to demonstrate binding of the receptor to the putative ligand- a primary requisite for hormone action! Analysis of the effects of all known hormones indicates that the two major groups discussed above do not explain everything. A third group of hormones/factors which can be called as 'Permissive' hormones which includes prolactin, glucocorticoid, thyroxine are eluding understanding with regard to how they permit other hormones to act. In their absence other hormonal effects are attenuated. What exactly is the molecular basis of permissiveness is not clear at the moment. No second messenger system is known to mediate its action. Prolactin receptors resemble cytokine and other growth factor receptor systems. In fact, metabolic hormones, developmental hormones, permissive hormones have to be studied together to understand extra cellular signal function. In another sense endocrine, paracrine, autocrine and kryptocrine regulation of cellular physiology can be understood as variations in the common theme of mechanism of communication between cells. Further, cells respond to physical (temperature, pressure, light etc.) & chemical (hormones, neurotransmitters, growth factors, pollutants etc.) stimuli. Integrative mechanisms appear to be similar in all these cases.

An interesting aspect of studying hormone action is viewing hormone action from an ontogenic and phylogenic perspective giving insights into evolutionary significance. In recent years the gene-knock out mouse model has given tremendous insights into hormone action [16].

Biosynthesis and regulation of secretion of hormones:

Comparison of endocrinology with communication engineering principles has demonstrated similarities like signal discrimination, signal transduction and signal amplification/dampening etc., but the basic question of what exactly is the signal in endocrinology has eluded satisfactory answer. Is it the hormone per se? Is it the concentration of hormone in the body fluids? Is it the concentration of receptor bound hormone? Is it the change in the circulating hormone concentration (i.e. amplitude)? Is it the frequency of such change? Not being clear in this, a currently accepted paradigm has been that change in the concentration of a hormone in circulation or bound to receptors constitutes the hormonal signal. This leads to the secondary question as to how this comes about. Is it affected by change in the rate of secretion from the endocrine gland, or in the rate of degradation in circulation/receptor bound/by non-target tissue? In order answer the question, measurement of hormone levels in circulation is necessary. Sensitive immuno assays have been developed [17]. Fluctuation in circulatory profile of hormones in a number of physiological states was noticed. While mammalian endocrinologists were relating acute changes in hormone levels to physiological processes, comparative endocrinologists were relating chronic changes over months if not days to reproductive physiological processes. This is an embarrassment to the theoreticians. Nevertheless this automatically led to studies on biosynthesis of hormones especially protein hormones.

In the case of small molecular weight (size) hormones, this was essentially classical biochemical work identifying the biochemical precursor and the enzymes which bring out the biochemical transformation. Metabolic studies were added later. In the case of epinephrine for example,, a simple amino acid like tyrosine serves as precursor. The enzymes carrying out hydroxylation, decarboxylation giving rise to dopamine, N-methylation have all been characterized well. Thyroxine is another example of an amino acid derived hormone. Tyrosine residues in thyroglobulin are iodinated and conjugated to give substituted thyronine residues. The details of iodide uptake by thyroid follicular cells, oxidation and organification have been worked out. However details of regulated proteolysis and secretion of hormone are not well understood.

The delineation of steroid hormone biosynthesis is one of the most important chapters in history of biochemistry. Metabolic labeling experiments had shown that cholesterol is the precursor of all the steroid hormones. Hence biosynthesis of steroid hormones was studied in two phases. Phase 1 involves biosynthesis of cholesterol from acetate. Isoprenoids are natural secondary metabolites derived from isopentenyl pyrophosphate, an intermediate from acetate to cholesterol. The biosynthesis of cholesterol can occur de novo in steroidogenic tissues and liver. Both de novo biosynthesized cholesterol and the plasma cholesterol taken up by the tissue can serve as precursors to steroid hormones. A series of hydroxylases, oxido-reductases, desmolases etc carry out all the steriospecific transformations to give rise to a spectrum of steroid hormones. In case of Sertoli cells and granulosa cells, aromatase comes into picture. This enzyme converts androgens like testosterone, andrast-4-ene-3, 17 dione into estradiol 17 β. The only hazy part of this steroidogenesis story is with regard to the mode of shuttling of cholesterol and other intermediates between mitochondria and endoplasmic reticulum. Star proteins have been implicated in this transport.

Among the small size hormones, peptide hormones ranging from a tripeptide like TRH, through octapeptides like vasopressin and oxytocin, decapeptides like GnRH, to polypeptides like MSH, ACTH, Insulin, Glucagon etc provide a fascinating story. The development of our understanding of the field had to await progress in the area of biosynthesis of protein hormones. Two general types of biosynthesis of peptides were known. One, where ribisomes are involved and another where ribosomes were not involved. The former involved genes, mRNA, tRNA etc. while the latter was a simple cytoplasmic enzymatic process linking amino acids one by one. Typical examples for the latter type were Glutathione and peptide antibiotics like gramicidin etc. In the case of the former type, details of biosynthesis of Insulin were worked out first, through conventional biochemical techniques like pulse-chase experiments etc. Later additional techniques like isolation of poly A rich RNA, in vitro cell free systems for translation, in vitro transcription assays, were used to understand the intricate details of protein and polypeptide hormone assembly by endocrine cells [18]. The stories of Insulin, Growth hormone, ACTH, PTH are major milestones in this field. The mystery of glycoprotein hormones of the thyrotropin family with regard to their mode of biosynthesis including that of the carbohydrates moiety have been worked out recently. It is interesting to note that work on protein hormone structure and biosynthesis has attracted half a dozen Nobel prizes.

Biosynthesis of polypeptide hormones:

The story of biosynthesis of Insulin, PTH, Endorphin, neurohypophyseal hormones, Glucagon and adenohypophyseal hormones is one of the most fascinating stories in molecular endocrinology. Molecular biology and Biochemistry have made significant contributions to this area of investigation. Pulse-chase experiments done on angler-fish pancreas initially gave rise to the idea that there is a single biosynthetic precursor for both the chains of Insulin. Subsequent developments in molecular biology permitted experiments like isolation of mRNA population by oligo-dT cellulose chromatography, *in vitro* cell-free translations using rabbit reticulosyte lysate or wheat germ lysate system etc. Such *in vitro* work revealed that insulin mRNA when translated gives rise to pre-pro-Insulin initially which is then processed by endoplasmic reticulum into pro-Insulin and then in secretory granules is finally converted into Insulin. A similar story was confirmed in the case of Glucagon, PTH and others. A general consensus was that the pre portion represents the 'signal peptide' which permits the hormone to cross the Endoplasmic membrane. Signal peptidase, present on the luminal side of the ER was supposed to remove the signal peptide to generate the mature protein hormone but still devoid of the tertiary structure. This is true for all exportable secretory proteins [19]. The details of the intra-cellular traffic of the nascent polypeptide precursor are being worked out in great detail. Using synthetic peptide as substrates enormous work has been done to understand the action and selectivity of signal peptidase but no consensus understanding has emerged except that the signal peptide portion is highly hydrophobic.

In the case of glycoprotein hormones like Luteinizing hormone (LH), Thyroid stimulating hormone (TSH) and Follicle stimulating hormone (FSH), two additional complications arose. One, they are glycosylated and hence one should know when, where and how they get glycosylated with a unique oligosaccharide specific to each of the hormones. Two, they consist of two non-identical and non-covalently linked subunits unlike in the case of Insulin. It was revealed soon that the individual subunits α and β subunits are synthesized on separate polysomes and translated from separate mRNAs**.** It was also revealed later that the disulphide bonds in the endoplasmic reticulum (microsomes as biochemists call it). Hence antibodies to the native subunits do not recognize the de novo formed pre- alpha and pre-beta subunits in in vitro translation systems. But antibodies to denatured subunits (reduced and carboxy-methylated) do recognize such de novo formed hormones.

The second part of the story dealing with the mechanism of specific glycosylation of LH, TSH, and FSH as also the specific combination and assembly of the common alpha subunit with different beta subunits in different cell types and sometimes in the same cell type (i.e. the FSH and LH secreting single gonadotrope) represents one of the most elegant investigations in cellular biochemistry. The carbohydrate of LH, FSH and TSH is of N-linked type while that of hCG and eCG (PMSG) are of both N-linked and O-linked types. Studies in late nineteen fifties revealed that individual sugars are always activated as nucleotide-sugars like for example UDP-Glucose or GDP-Mannose etc. In seventies it was shown that there is a common 'lipid linked oligosaccharide' intermediate for all N-linked oligosaccharides independent of their final structure which differs from hormone to hormone [20]. This lipid (Dolichol) linked oligosaccharide had a molecular formula i.e. GlucNAC2 Man9 Gluc3.

This common intermediate structure is en mass transferred onto an incoming de novo synthesized protein within the ER. This step is sensitive to inhibition by protein translation inhibitors like cyclohexiimide and hence is called Co-translational event. Subsequently during the sojourn of the glycosylated protein through the Golgi apparatus, peripheral sugars are processed and replaced to give rise to the final but individual pattern of glycan structure in each of the three

pituitary hormones and the placental hormones. These events in the Golgi apparatus are called post-translational modifications.

How are these Co-translational and post-translational events including assembly of the heterodimers coordinated and regulated is not very clear. This problem can be appreciated knowing that a single gonadotrope can secrete both LH and FSH. As α subunits are common to all the hormones in a given species, the regulation of content of each mRNA and each gene product becomes a difficult task in such cells. With new techniques available, this problem is being investigated. Northern blot analysis has demonstrated fluctuations in mRNA levels in pituitary gonadotropes paralleling the changes in circulatory levels of the hormones. However in some cases such correlations were not observed. In the case of the O-linked oligosaccharide the individual sugars are activated and linked in a series of enzymatic reactions. What is not clear is the mechanism of the sugar addition sequence determination and also the fidelity of this glycosylation.

The presence of free subunits in circulation and even in pituitaries has been unambiguously established. The inability of such freely occurring subunits to combine to form the heterodimeric hormone has been attributed to hyper- as well as hypo glycosylation. The biological significance of these freely occurring hormone subunits is being worked out. The chromosomal location of the genes for these subunits has been established. Indeed both the genes and their corresponding mRNAs have been fully characterized.

Structure sub serves function?

Biological information has to be viewed from the point of 'theory of evolution by natural selection' to make sense. Though reductionist biology attempts to explain phenomena by the concepts of physics and chemistry, it does not satisfy an organismic biologist [21].Reductionist biology merely talks of mechanisms i.e. proximal causes and their effects. One of the major concepts in evolutionary biology is that STRUCTURES HAVE EVOLVED TO SUIT A FUNCTION. This permits discussion of target of natural selection. There must be a feature which gets selected by Nature and has thus evolutionary or survival advantage. At the molecular level also this debate can be raised and one can legitimately ask what structural features of hormones are those that are selected for better hormonal activity. To answer this question, one should first of all study the physico chemical structure of all the hormones, have a comparative perspective and then try to answer this vexing question.

Hormones exhibit a variety of structures ranging from simple amino acid derivatives through steroid like structures to polypeptides and huge glycoproteins. Our knowledge of the structural details is almost complete. Hormones like epinephrine and thyroxine are simple derivatives of Tyrosine. Hormones like TRH, LHRH, and ACTH etc., are simple peptides. Hormones like Prolactin and Growth hormone are long polypeptides while LH. TSH, FSH, hCG and PMSG are glycoproteins. Steroid hormones are lipid soluble and water insoluble. The diversity of hormonal structures, a priori, indicates that there may not be a chemical clue to hormonal activity. Indeed, over hundred years of endocrinology has not come up with a 'consensus' structure for possessing hormonal activity. Hence the closest that one can come to, is to understand the role each part or functional group of the structure in the hormonal activity. Thus one can talk of the importance of the aromatic ring A for estrogenic activity. Similarly one can talk of the importance of key amino acid or side chain group like guanido/ phenolic group for hormonal activity in a protein hormone. This does not answer the basic question of what is the structural basis of 'hormonal activity'. Notwithstanding this fact that we cannot understand the chemical uniqueness of hormonal structures, enormous research work has been done on the structures of hormones. Prior to determining the structure of any hormone, one has to isolate the hormone in a homogeneous state or purify it. Purification of hormones has been done for over seven decades. Criteria for purity have been established.

One of the fundamental features of protein hormones, especially the glycoprotein hormones, is their structural micro heterogeneity. This simply means that preparation of the hormone, that contains only that particular hormonal activity, when checked by a vastly more sensitive technique exhibits multiple components. What is the biological significance of these micro heterogeneous isoforms of the same hormone? One interesting hypothesis suggested has been that the micro heterogeneous isoforms have differing potency in a given bioassay during ontogeny and that during phylogeny they may exhibit different biological activities [22]. Another crucial aspect of the same problem is to find the stoichiometric relation between hormone and its cognate receptor. Is it 1:1? If it is 1:2, it raises the question of anisotropy in the hormone. Thus the structure of the complex should be determined rather than the hormone alone or the receptor alone.

In the case of the protein hormones, there are two approaches to the understanding the structure-function relationship: One, antigenic epitope analysis using monoclonal antibodies and using receptor binding/response inhibition studies to identify a particular epitope in the hormonal structure involved in the interaction with the receptor. Later a synthetic peptide is used to simulate this epitope. The second

approach is to determine the structure of the hormone-receptor complex as mentioned earlier. Either way the information generated has tremendous applications. Synthetic peptides which can mimic or inhibit a given hormone action are very useful biopharmaceuticals. At least in reproductive biology, the best contraceptive would be a receptor antagonist to LH or FSH. One can then extend this idea to natural products that have the similar structure. Morphine is a classic example. Modulation of hormone secretion or action by natural or synthetic compounds is a very exciting area of research in molecular endocrinology. The work so far done in endocrinology suggests that we know so much about hormone structures but still may not get any clue to the hormonal activity. This would raise the crucial question whether function (receptors) adapted to preexisting hormonal structures (as signal) or the other way? Evolution of hormones and their cognate receptors is an incomplete but exciting chapter in molecular endocrinology.

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