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0014-4754/86/070782-07$1.50 + 0.20/0

Development of hormone receptors: Conclusion

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Key words. Electrostatic complementation; evolution; genetic code; hydropathic complementation; receptor imprinting.

When I was asked to write the conclusion to this multiauthor review, I was somewhat reluctant at first, since the biochemistry of hormone receptors is far from my own field of research. However, I accepted this invitation because it is my personal experience that the view across borders into neighboring fields can bring new insight into one's own field of endeavor. I apologize if my sometimes unorthodox way of thinking about scientific problems and questions may now and then challenge traditional points of view, but possibly this is the true reason why I was asked to write this concluding review. Scientific data are traditionally looked at with the attitude that *anything that cannot be proved does not exist.* Historical development, however, has repeatedly shown that the attitude that *only those things which can be disproved do not exist* may hit the truth much better.

After reading these ten highly interesting expert reviews on specialized questions of hormone and receptor structure and function, phylogeny and ontogeny, imprinting and recycling, it would be most presumptuous of me to claim to present a final answer to the many open questions on how receptors may arise. Therefore, all those who expect the elaboration of a textbook-style theory on receptor development, based on classical points of view, will be disappointed. Research on receptor development is not ready yet for a classical theory. Classical theories give the impression that everything is already known and, owing to this impression, they inhibit the development of new insights and further research. *Man makes theories, but nature does as it very well pleases.* And because nature does as it pleases, a conclusion is nothing else but *the point where one gets tired of thinking.*

Even in science it is easier to swim with the stream (which scientist has not yet had this experience, especially when making a grant application?), but if you want to get to the source then you must frequently swim against the stream. So please forgive me, if I sometimes leave the treadmill of traditional thinking during my vague attempt to tie up some of the open ends. Remember, *you cannot pass someone, if you only keep stepping into his footsteps.*

Vertebrate hormones exist in invertebrates, in unicellular organisms and in plants

The cells of the body communicate with each other through biochemical mediators, like hormones, neuroactive substances, paracrine agents and pheromones. Previous reviews[3, 11, 35, 42] have demonstrated that this particular method of communication existed already very early in evolution and has survived for hundreds of millions of years. Throughout these years a great number of mutations have generated new phyla, new classes, new orders and an enormous number of species with immense inter-individual variability.

Is it not surprising how stable the structures of biochemical mediators are? They have survived millions of mutations from unicellular organisms to mankind. Vertebrate hormones have been detected in non-vertebrate animals, in plants, and even in unicellular organisms. Insulin, for example, has been identified in insects[19, 38], annelids[38], and molluscs[57], in a variety of unicellulars[43, 44, 60], and in plants like spinach, lemna and rye (for a review see ref. 42). Neurotensin[5], somatostatin[4, 39], ACTH[40], β-endorphin[40], relaxin[62], and calcitonin[16, 55] have been identified in various unicellular organisms.

Although the universal distribution of these hormones has been well documented, very little is known yet about the function of vertebrate hormones in lower organisms. Insulin might universally be involved in carbohydrate metabolism; its involvement has been demonstrated not only for vertebrates, but also for insects[56] and molluscs[57]. Similarly, the pituitary hormone ACTH and the opioid β-endorphin may not only be involved in the stress response of vertebrates, but also in the stress response of other organisms, since the stress response is a very universal phenomenon for adaptation. Then how about the function of opioids in wheat[70]? Is it imaginable that plants respond to stress with a hormone reaction similar to that of animals? The identification of interferon in tobacco[63] raises the question whether plants may respond to viral infections in the same way as animals. With the best efforts of my imagination, however, I cannot suggest a function for the pregnancy hormone human chorionic gonadotropin (hCG), which has been identified in bacteria[1, 49], or for luteinizing hormone-releasing hormone (LHRH), which has been extracted from oak leaves[21], not to mention the presence of somatostatin in spinach, lemna and tobacco[41] and the presence of thyrotropin-releasing hormone (TRH) in alfalfa vegetable[27, 51].

Lower organisms possess receptors for vertebrate hormones

What is the functional significance of vertebrate hormones in lower organisms? This question will certainly give rise to some very interesting research projects in the future, but at present we lack possible answers. Instead, for the time being, we must be content to search for answers to the question whether or not lower organisms actually possess binding sites or receptors for vertebrate hormones.

This question was reviewed by LeRoith et al.[42]. The authors listed a number of examples of the existence of receptors for vertebrate hormones in lower organisms. Estrogen receptors and corticosterone receptors were observed in different species of yeast[8, 45, 46], opiate receptors were localized in amoeba[30], and binding sites for TSH were observed in a number of bacterial species[68]. The presence of receptors for vertebrate hormones in non-vertebrate organisms supposes that these receptors may have physiological meaning for these organisms. In addition, these receptors may be of important clinical relevance. Infection through bacteria, which carry receptors for human hormones, may generate an immune response in humans against the bacterial receptors, and may then encroach upon the inherent endogenous human receptors. Heyma, Harrison and Robins-Browne (cited by ref. 42) suggested the possibility that bacterial TSH-binding sites may generate antibodies in humans which cross-react with the endogenous TSH receptors of the human thyroid gland. These antibodies may activate the production of thyroid hormones in the thyroid cells, which will result in hyperthyroidism. The authors observed that binding of TSH to TSH-receptor carrying bacteria was inhibited by sera from patients with Graves disease, but not by sera from other people. Thus, the study of endocrine phylogeny is not only of comparative interest; it may be of great importance for the clinical endocrinologist as well.

The multifold existence of receptors for vertebrate hormones in lower organisms of different phyla gives rise to the question whether actually each individual organism in this universe may already possess the whole array of hormone receptors known to exist in vertebrates. The fact that binding sites for human chorionic gonadotropin were observed in *Pseudomonas maltophila,* but not in other microorganisms, such as *Psdeudomonas aeruginosa* and other gram negative rods[59], indicates that the development of a particular hormone receptor is a very specific affair for each individual species (or individual organism?). On the other hand, it was already suggested previously[18] that sometimes methodological problems may mask the true results. Thus, *the inability for a particular method to detect a certain parameter is no certain proof of the non-existence of this parameter.* Frowein et al.[20] demonstrated that gonadotropin receptors exist in gonadotropin-insensitive Leydig cells of immature rats; however, they are present in a 'masked' form, and they bind to gonadotropins only after they have been 'unmasked'.

Evolutionary aspects of hormones and receptors

The fact that vertebrate hormones and their receptors have been detected in lower organisms does not necessarily mean that their chemical and physical structure as well as their biological activity is fully identical in all organisms from unicellulars to humans. True comparisons are usually difficult to make, because of the profound differences in organization and mode of life between widely separated taxa[2]. It was mentioned previously that some of the same peptide hormones can be detected in vertebrates, invertebrates, unicellulars and even plants. The chemical and physical structure of peptide and polypeptide hormones, however, has in many cases undergone diversification throughout phylogeny.

These diversifications can arise at the level of the genetic code by point mutations within the DNA, resulting in specific amino acid replacements within peptide hormones[3]. Insulin molecules, for example, differ in different species with respect to the number and position of amino acid substitutions. After 500 million years of independent evolution the insulin molecule of the hagfish differs from that of man in approximately 38% of its amino acids[52]. Nevertheless, the different insulin molecules of different species share common biological properties[66].

Diversification of peptide molecules throughout evolution raises the question whether or not the receptors for these hormones may, in parallel, have undergone equivalent diversification. This question has been studied in detail by extraction and characterization of hyperglycemic hormones from several crustaceans, including an isopod and two decapods[48]. These hormones were shown to be peptides with 50–58 amino acids, with overall similarity of composition, but with much interspecies variation in amino acid substitutions. Cross-reaction studies indicated that the receptors have varied side by side with the hormones[3,48].

As mentioned in the previous paragraph, hormones may undergo structural alterations during evolution without changing their biological properties. As reviewed in detail by Barrington[3] hormones of common ancestry may also undergo functional diversification during evolution and may eventually establish new target relationships. Such a case was discussed by Barrington[3], concerning the structural resemblance of 4 invertebrate peptide hormones with different biological functions. The 8-residue erythrophore-concentrating hormone of crustaceans, the 10-residue adipokinetic hormone of insects and the two 8-residue peptides periplanetin CC-1 and periplanetin CC-2, which both show adipokinetic activity in grasshoppers and hyperglycemic activity in cockroaches[61], are sufficiently alike structurally to imply divergence by point mutation from a single ancestral molecule[3,50]. Similarly, the two neurohypophyseal peptide hormones vasopressin and oxytocin have different biological functions, although they are both derived from the common ancestor molecule arginine vasotocin. Arginine vasotocin exists in fish, amphibians, reptiles, and birds, but not in mammals. Finally, a particular hormone from one species may be totally inactive in another species. Human growth hormone, for example, stimulates growth in humans and monkeys. Bovine growth hormone stimulates growth in the bovine, but it is ineffective in humans and monkeys. Similarly, mammalian gonadotropic hormones, even in tremendous doses, do not stimulate the gonads of certain amphibian species, whereas relatively small amounts of amphibian pituitary extracts will do so[22].

Sometimes a pedigree of endocrine phylogeny can be established. As reviewed by Barrington[3], the two mammalian pituitary hormones somatotropin (growth hormone) and prolactin have some 23% of their residues in common, which is generally conceded to indicate divergence from a common molecular ancester. Immunological evidence has shown that growth hormone and prolactin are already present in the lamprey's pituitary gland, at the beginning of vertebrate evolution; this indicates that the two hormones must have diverged very early during evolution. A third member of this hormone family, human placental lactogen (hPL), shares some 85% of its amino acid residues with human growth hormone. This great similarity indicates that hPL must have diverged from growth hormone much later, probably during primate evolution[67]. During the long period of prolactin evolution, this hormone has established a great variety of target relationships. Just to mention a few examples, prolactin maintains luteal function and initiates milk secretion in mammals, it stimulates broody behavior and the production of crop milk in birds, it stimulates behavioral water drive in terrestrial salamanders, and it stimulates nest-building, melanogenesis and osmoregulation in some fishes[22].

The genetic code for receptor synthesis

We have seen from previous reviews[3,11,35,42] that there are hormones which existed very early in evolution and which remained structurally and functionally rather stable. Other hormones underwent structural modifications, but their biological functions remained the same. A third group of hormones remained structurally rather stable, but their biological property changed during evolution. The change in biological activity sometimes went in parallel with the necessity for the species to adapt to a new environment, or with the phylogenetic development of new organ systems, which then became the new target for an old hormone, phylogenically speaking. Some hormones, finally, have changed during evolution in both structure and function.

In any case, the evolutionary development of a new hormone, or the mutational alteration of an already existing hormone, must go in parallel with the development of a new specific receptor, or with the structural adaptation of an already existing receptor. This new receptor must be able to recognize and interact with the new hormone in order to elicit a biological function. Teleologically speaking, it would be most efficient for nature if the same mutation which generates a new hormone could elicit a complementary mutation, which provides the specific receptor.

In this regard an interesting pattern in the genetic code was recently observed. Codons for hydrophilic and hydrophobic amino acids on one strand of nucleic acid are complemented by codons for hydrophobic and hydrophilic amino acids on the other strand[6]. The average tendency is for codons for 'charged' amino acids to be complemented by codons for 'uncharged' amino acids. Following this pattern, two peptides that represent complementary strands of nucleic acid will display an interchange of their hydrophilic and hydrophobic amino acid residues when the amino terminus of one peptide is aligned with the carboxyl terminus of the other. For receptor-hormone interaction and binding to occur, the two structures have to be complementary with respect to their hydrophobic and hydrophilic domains. One possible consequence of this observation is suggested by the finding that many biologically important peptides, composed of 10 to 50 amino acids, can assume amphiphilic secondary structures in the presence of another amphiphilic structure such as a membrane or a receptor binding

site[31]. As a result of this relationship Bost et al.[7] hypothesized that complementary DNAs, when transcribed in the 5′ to 3′ direction and in the same reading frame, will code for peptides or proteins that interact. This hypothesis was tested by the authors, and they demonstrated that the two naturally-occurring peptides corticotropin (ACTH) and gamma-endorphin bind specifically and with high affinity to synthetically derived counterpart peptides, which had been specified by RNA sequences complementary to the mRNA for ACTH and gamma-endorphin, respectively[7].

DNA molecules resemble chain ladders, twisted into a helix, in which pairs of bases join two linear chains constructed from deoxyribose and phosphate subunits. The bases invariably pair so that adenine links to thymine and guanine links to cytosine on the complementary DNA strands. Adenine, thymine (which is replaced on RNA by uracil), guanine and cytosine act as code letters, ultimately for the recognition of particular amino acids during ribosomal peptide synthesis. Due to the complementary structural relationship between the two DNA strands, a mutation on one strand would inevitably elicit a complementary mutation on the other strand. In other words, a point mutation occurring in a particular section of one DNA strand, which encodes for a particular peptide hormone, will result in the substitution of one amino acid in this particular peptide molecule. If the substituted amino acid has different hydrophobicity or hydrophilicity as compared to the previous amino acid, then the peptide will assume a secondary and tertiary structure different from that of the premutational peptide. Since the point mutation has altered one partner of one pair of bases, let us say adenine was replaced by cytosine, then thymine as the other partner of this base-pair must be replaced by guanine in order to ensure cross-linkage of the two complementary DNA strands. As a natural consequence the transcription of the mutated complementary DNA strand would encode for the synthesis of a peptide receptor which has acquired one substituted amino acid. This substitution may determine the secondary and tertiary structure of this new receptor molecule in such a way that it can interact with the new peptide hormone.

Another way in which evolution may have generated the primordial receptor of a peptide is by translocation of the peptide's complementary DNA sequence.

Hormone-induced activation of receptor synthesis

In every multicellular organism the genetic code of this organism is present in each one of its individual cells. Activation of the genetic code, however, must occur differently in different cells, because during cell differentiation each cell is known to acquire its very specialized structure and function. Transcription of both strands of DNA of a particular gene in the same reading frame is known not to take place within the same cell. Considering receptor-hormone interaction, it would not make sense, teleologically speaking, if the hormone and its receptor were to be synthesized within the same cell. A hormone is, by definition, a chemical mediator which delivers information to distant cells, via the extracellular fluid. If the hormone and its receptor were synthesized within the same cell, then the specific affinity of the receptor for the hormone would not allow the hormone to leave the cell. Therefore, a mechanism must be in operation, which activates transcription of a particular DNA sequence in one group of cells (i.e. the endocrine gland) and activates transcription of the complementary DNA sequence in another group of cells (i.e. the hormonal target organ). This mechanism would allow for specific cellular recognition and for distant communication via the interacting peptide products.

The mechanisms by which transcription and translation of the genetic code are activated, are still very obscure and leave plenty of room for speculation. Hormones and/or receptor-hormone complexes may very well be involved in activation of the genetic code. DNA binding by steroid receptors has been a popular topic for many years[69]. After activation by estrogens, estrogen receptors are known to bind tightly to DNA[24]. As mentioned in the review by Gorski[23] it was shown recently that progesterone receptors[53] and glucocorticoid receptors[32, 54] bind to very specific sequences of DNA, which are known to be essential for the expression of progesterone and glucocorticoid action, respectively.

Estrogenic interaction with the genetic material in the cell nucleus was shown to stimulate DNA synthesis and estrogen receptor resynthesis in the cytosol[28, 29]. Estrogens were also shown to stimulate the synthesis of progesterone receptors[17, 33, 34]. The time course of this induction suggests that a transcriptional site of action is involved[23]. Posner et al.[58] observed that estrone and estradiol induced lactogenic receptors in rat liver membranes via a pituitary mechanism. The receptors were able to bind ovine prolactin and human growth hormone with high affinity. Hypophysectomy drastically decreased the levels of lactogenic receptors in mature female rats and in these animals estrogen failed to restore receptor levels[58]. Thus, a factor released by the pituitary gland (growth hormone?, prolactin?) is primarily responsible for the induction of lactogenic receptors.

Hormone-induced organization of receptors

A great number of studies, performed with unicellular organisms, seems to indicate that the extracellular hormonal environment of a cell can determine the development of receptor systems in this particular cell[11, 35]. Serotonin and histamine, the phagocytosis-stimulating hormones of higher organisms, also stimulate phagocytosis of the ciliated unicellular organism *Tetrahymena*[13]. Insulin and adrenalin stimulate glucos metabolism, thyroxine stimulates growth, and thyrotropin (TSH), follicle stimulating hormone (FSH) and ACTH stimulate RNA synthesis in *Tetrahymena*[10]. The first encounter of *Tetrahymena* with the hormones mentioned above was shown to enable the organism to bind these hormones to a greater extent on the next occasion[36], and the response of this organism to the particular hormone increased on reexposure[9]. Antibodies raised against rat hepatocellular insulin receptors were shown to bind to *Tetrahymena* cells which had been pretreated with insulin, to a significantly greater extent than to untreated control cells[12]. From these studies, it appears that the first encounter of

Tetrahymena cells with insulin generated insulin-receptors which were immunologically similar to the insulin receptors of the rat hepatocellular membrane. This conclusion is supported by the observation that treatment of rat hepatocytes with antiserum, raised against insulin-pretreated live *Tetrahymena* cells, inhibited the insulin binding capacity of rat hepatocytes to a greater extent than antiserum raised against untreated *Tetrahymena* cells[37]. The authors concluded that the insulin receptors of the *Tetrahymena* are not preformed structures, but seem to arise under the influence of insulin[35].

As the result of a great number of studies with various hormones, Csaba and his associates came to the conclusion that hormonal presence is an indispensable prerequisite of receptor formation[9, 11, 35]. The first encounter of a particular hormone with a primordial binding site will result in the 'imprinting' of this binding site into the complementary shape of the hormone. This mechanism of imprinting will permanently increase the binding affinity of the primordial binding site to the hormone. The imprinted receptor will 'memorize' the shape of the hormone throughout many cell divisions. In *Tetrahymena* this 'memory' for the binding of insulin was still demonstrable after as many as 500 cell generations[11]. However, this 'memory' will eventually fade if the receptor is deprived of repeated contact with the hormone[11]. The mechanism of 'fading receptor memory', or receptor desensitization, as it may also be called, is well known to occur in situations where a particular hormone has been removed from the circulation. The receptor remains sensitized only as long as it has repeated (sometimes pulsatile) contact with the hormone. In case of excess hormonal activity, membrane receptors may actually be 'down regulated', possibly as a result of endocytosis, thus avoiding hyperreaction of the cell.

Primordial binding sites may be located as proteins or glycoproteins within cellular membranes, or they may be dissolved in intracellular (cytoplasm) or extracellular (plasma) fluid. Receptor imprinting has not only been demonstrated in unicellular organisms, but also in mammalian cell lines and in multicellular organisms during development[9, 11]. Insulin induced imprinting of insulin receptors in mammalian cell lines at a very low concentration (10^{-13} M) and during the rather short exposure time of only one hour[14]. Similarly, TSH and FSH were able to imprint their receptors in Chinese hamster ovary cells in cell cultures. The authors[15] observed that the receptor imprinted by TSH was able to bind both TSH and FSH. The receptor which had been imprinted by FSH was also able to bind either hormone. This type of overlap in the imprinting effect was attributed to the structural similarity of the two glycoprotein hormones, which are known to share a common alpha subunit.

A great number of experiments have been performed on the imprinting effect of steroids, peptides, amino acids and cardioactive glycosides during pre- and postnatal development of rats and chickens[11]. From the results of these studies, the authors concluded that, even in higher organisms, imprinting of receptor systems is result of a first contact with the particular hormones. This imprinting, however, depends on particular sensitive phases during development of the organism and it depends on the concentration of the interacting hormone. The appropriate hormone can actually damage the receptor permanently if it appears at an unsuitable time and at an inappropriate concentration[11]. A similar conclusion was drawn by Döhler[18] in regard to sexual imprinting.

The observation that hormone-induced receptor imprinting is transmitted from one cell generation to the other, and was demonstrable in *Tetrahymena* after as many as 500 cell generations[11], indicates that hormonal imprinting of receptors is not only a mechanism limited to the cell membrane. Normal metabolic turnover of the membrane is so rapid that membrane-associated structures are replaced very rapidly. Csaba[10] concluded from his studies that receptors can be defined as genetically determined structures whose expression is subject to amplification by environmental influences. Amplification does not take place in isolated (hormone free) conditions.

Teleologically speaking, the synthesis of a hormone and its receptor within the same cell does not make sense. Hormones and receptors only interact during a short period of information exchange. For example, cell A releases hormone A, which carries a message for cell B. In order to understand the message, cell B has to develop a specific receptor for hormone A. If cell B wants to reply to cell A, then cell B has to release hormone B and cell A has to develop a receptor for hormone B. A very clear example for this type of interaction was given by LeRoith et al.[42] in their description of mating factors in the budding yeast *Saccaromyces cerevisae*. This yeast exists in a diploid form as well as two haploid forms, designated 'alpha' and 'a'. Union of two haploids of opposite type is a process which requires two peptide pheromones or mating factors, 'alpha' factor and 'a' factor. 'Alpha' factor is produced by 'alpha' cells and acts only on cells of the opposite type ('a' cells), via specific cell surface receptors. 'A' factor is produced by 'a' cells and acts only on 'alpha' cells, via specific cell surface receptors[64]. Interestingly, 'alpha' factor bears a striking structural resemblance to mammalian gonodotropin releasing hormone (GnRH), binds to GnRH receptors of rat pituitary cells, and causes release of gonadotropins from the pituicytes[26, 47].

Another example, mentioned by LeRoith et al.[42], demonstrates the organizing effect of a hormone from one cell on the target organ of another cell. In the unicellular fungus *Achlya ambisexualis* the female secretes antheridiol, a steroid pheromone, which induces in the male the formation of antheridial branches (sex organs) and the secretion of oogoniol, a second pheromone. Oogoniol causes the female to develop sex organs[25].

A teleological point of view

The development of a receptor for a particular substance is necessary for a cell, teleologically speaking, only if uptake of this substance as an information carrier has a selective advantage for the cell. Vertebrate hormones are normally not present in the extracellular environment of unicellulars, and therefore there is no need for this organism to develop receptors for vertebrate hormones. If, however, the composition of the extracellular environment should change, then it may become advantageous for the organism to develop immediately specific receptors for the substances in the new environment. It would

be very inefficient for the organism to wait for the remote chance of an accidentally occurring genetic mutation which, through the supply of a new receptor, would help the organism to adapt to the new environment. The predisposition of 'plastic' primordial receptors through genetic mechanisms and the final shaping (imprinting) of these receptors, depending on the particular environmental needs, would indeed be a 'fail-safe' mechanism through which nature provides optimum adaptability to changing environments. The same system would also work for multicellular organisms. During sex determination, for example, there is a genetic predisposition towards either male or female. For final development of organ structure and function, however, this genetic predisposition can be overruled by the priming action of circulating sex hormones[18].

During early development of an organism the shape of primordial membrane receptors may be oriented in line with neighboring structures of the cell membrane, thus forming a yet undifferentiated unit of optimal molecular energy distribution. One may envision a process in which the initial contact of a hormone with a primordial receptor is sufficient for recognition of the appropriate future binding site. After this recognition considerable induction of shape may take place, during which the electrostatic forces of the hormone and its hydropathic composition will force the receptor to take a shape providing complementary electrostatic forces and complementary hydropathic form. The mapping of electrostatic potential onto molecular surfaces has revealed the importance of electrostatic forces in intermolecular interactions[65]. A similar mechanism has been suggested for the induction of antibodies by antigens[65].

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