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CENTRAL CONTROL OF PROLACTIN AND ESTROGEN RECEPTORS IN RAT LIVER—EXPRESSION OF A NOVEL ENDOCRINE SYSTEM, THE HYPOTHALAMO-PITUITARY-LIVER AXIS

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INTRODUCTION

The many and complex functions of the liver require extensive control mechanisms. Consequently, several liver functions seem to be regulated by the endocrine system. The initial event by which most hormones exert their effects is via association with a specific receptor present in the target tissue (for review see 1, 2). These hormonal receptors are characterized by a narrow ligand specificity and a high-affinity, low-capacity binding. Receptor

proteins occur as soluble species in the cytoplasm or nucleus of the cell and can also be found as hydrophobic proteins embedded in the plasma membrane of the cell (for review see 3, 4). In the case of the liver, a large number of different types of receptors have been detected. This review focuses on central control mechanisms regulating hepatic prolactin and estrogen receptors.

Regulation of Hormone Receptors

The knowledge of hormone receptors was greatly increased by the development of radiolabeled ligands. An important discovery was that the number and/or affinity of the receptors in the target cells is not fixed, but that a dynamic state exists in which several factors may influence the concentration and/or ligand affinity of a receptor (for review see 5). The peptide hormone receptors in particular have been extensively studied in this regard. "Down regulation" seems to be a general phenomenon with these receptors. This situation, in which the ligand reduces the concentration of its own receptor, has been shown for example with the insulin receptor (6) and with the growth hormone receptor (7). A hormone receptor may also be influenced by hormones other than its own ligand; e.g. it is known that FSH can increase the concentration of the LH receptor in the ovary (8). Regulation of hormone receptors probably represents a mechanism important to the organism in regulating its sensitivity towards hormonal signals. It has also been suggested that receptor regulating mechanisms may be disturbed in certain pathological conditions (for review see 9). Usually, the most important factors involved in regulation of hormone receptors seem to be the hormones themselves (for review see 10). Therefore, it is important to consider serum concentrations of circulating hormones as well as the control of hormone release into circulation when studying hormone receptor regulation.

Regulation of Release and Properties of Pituitary Hormones Affecting Liver Functions

The secretion of pituitary hormones is regulated by the central nervous system (CNS) (for review see 11). In case of the anterior pituitary hormones, neurohormonal substances are released from the median eminence and transported to the pituitary via a portal blood system. The pituitary hormonal secretion is influenced by inhibitory and/or releasing hormones. Of the six well defined hormones secreted by the anterior pituitary, this discussion will concentrate mainly on growth hormone (GH) and prolactin (Prl). GH and Prl are polypeptide hormones with molecular weights of about 20,000–23,000. Structural similarities between these hormones suggest a common ancestral gene. It has been possible to isolate and sequence

both GH and Prl from several species (for review see 12). However, recently evidence has been presented that both GH and Prl actually consist of several "isohormones" with minor structural differences (13, 14). This hormone heterogeneity may have several origins; in the case of GH, various enzymatically modified forms of the protein that may reflect enzymatic processing in vivo have been described (15, 16). In the case of hGH, one of the many hGH variants has been studied particularly well. This species, called the 20 K hGH (17), lacks 15 amino acid residues and thus has a lower molecular weight than normal hGH (22 K). Analysis of the structure of the hGH gene makes it likely that one possibility for the occurrence of the 20 K variant is the result of a duplicated gene, in which an intervening sequence (intron) has been lengthened to include part of a coding sequence (exon) (18). Gel filtration of serum indicates that GH and Prl exist with different molecular weights in the circulation (19, 20). The biological significance of the different forms of the hormone is unclear.

Prl was first isolated from sheep pituitary glands in the 1930s (21), and human Prl was purified as late as in 1972 (22). Structural analysis has demonstrated that the amino acid sequence of the polypeptide varies to a certain extent between different species (23). The total number of amino acids is 198. The secretion of Prl is predominantly regulated by a hypothalamic inhibitory hormone, possibly identical to dopamine (24). In the CNS, the mediobasal hypothalamus is an important regulatory site (25). The best known function of Prl is its stimulation of lactogenesis (for review see 26). In addition, a large number of other functions of Prl have been described in mammalian and submammalian species. Prl has been reported to participate in reproductive, metabolic, and developmental functions as well as in regulation of electrolyte balance (for review see 27). The cellular mechanism of action of Prl is not clear. It may involve alterations in cyclic nucleotides, influence on prostaglandin synthesis, stimulation of polyamine synthesis, or changes in intracellular sodium and potassium concentrations (for review see 28). Serum levels of prolactin may be measured by radioimmunoassay (RIA), and an episodic secretory rhythm has been found in humans. Women tend to have a somewhat higher and more variable serum level of Prl than men. A marked increase of serum Prl is seen during pregnancy (for review see 29). In rats, Prl is also secreted episodically (30) and females tend to have higher serum levels of Prl, particularly during estrus and during lactation (31).

GH was first isolated by Li et al (32) and its amino acid sequence (191 amino acids) is now known for several species (12, 33). The hypothalamus regulates GH secretion in a dual fashion: the inhibitory hormone is the tetradecapeptide called somatostatin (34), and available data also support the existence of a releasing hormone (35). In the rat, the inhibitory influence

on GH secretion originates from anterior hypothalamic structures (36). A releasing "center" could possibly reside within the ventromedial hypothalamus (37). GH has long been recognized as an anabolic and growth promoting hormone with actions on several different tissues (for review see 38, 39). The increase in skeletal growth rate following GH administration is correlated to the production of a peptide (somatomedin) by the liver (40, 41). Other actions on the liver exerted by GH are the stimulation of amino acid uptake and protein synthesis (42); an increased synthesis of hepatic RNA has also been shown following GH administration (43). The mechanism of action of GH in the liver is largely unknown. However, the initial event is probably binding to a membrane-bound receptor (44). It has also been suggested that GH alters the activity of ribosomes, and several possibilities have been presented as to how this effect may be caused (45).

Serum levels of GH, as measured by RIA, show large fluctuations in both humans and rats (46, 47). In the rat, sexual differences exist with regard to pituitary GH content (48) and in the episodic release of GH. Whereas male rats show regular surges of GH with 3–4 h intervals, female rats display less regular surges with a higher baseline between peaks (49).

In addition to GH and Prl, other pituitary hormones affect the liver, e.g. ACTH (mainly via the adrenals) and TSH (via the thyroid) (50, 51). The secretion of these two hormones are in part regulated by the hormones produced following stimulation of the respective endocrine glands (feedback inhibition) (for review see 52). In case of GH and Prl, no such feedback inhibition exists with certainty. However, as is the case with other pituitary hormones, the secretion of GH and Prl is influenced by numerous factors. Besides hormonal stimuli, environmental and nutritional factors for example may also affect pituitary hormonal secretion.

In conclusion, several well-known pituitary hormones participate in the control of liver functions. An example of a hepatic function regulated by the pituitary is the sexually differentiated drug and steroid metabolism. In this case, the responsible pituitary factor did not seem to be identical to any of the known pituitary hormones (53–55). This led to the postulation of the existence of a pituitary hormone, the "feminizing factor." This factor feminizes drug and steroid metabolism in rat liver and is regulated by inhibitory signals from the anterior hypothalamus (for review see 56).

Sexual Differentiation of Prolactin Receptors in the Liver

In the rat, Prl binds to several different tissues, i.e. mammary gland, liver, ovary, kidney, prostate, and brain (57–63). This membrane-bound hormone receptor has been partially purified from rabbit mammary glands and its molecular weight was determined to approxmately 220,000. An antiserum has been developed against the Prl receptor (63, 64). Also, the Prl receptor

in rat liver has been partially purified (65). Although the function of the hepatic Prl receptor is not clear, it has been reported that Prl stimulates liver RNA synthesis (66) and causes an increase in ornithine decarboxylase activity (67) and thymidine kinase (68). Other claimed effects of Prl on the liver are an increased somatomedin production (69) and an increase in the concentration of estrogen receptors (70). Whereas male rats show a low or nondetectable level of Prl receptors, female rats bind Prl with high affinity and low capacity. This sexual differentiation of the hepatic Prl receptor concentration develops at the time of puberty (58). Thus, the hepatic Prl receptor constitutes a nice model for studies on receptor regulation and, consequently, the endocrine control of liver Prl receptors has been extensively studied (for review see 71). Ovariectomy reduces and subsequent estrogen administration increases Prl receptors to a level found in intact female rats (72). When male rats are castrated, a slight increase in Prl receptor concentration is noted, an effect that is reversed by androgen administration (73). Furthermore, the presence of Prl receptors in female rats can be reduced by androgen treatment (74). These results indicate that gonadal steroids are important in regulation of hepatic Prl receptors. In addition, the pituitary gland occupies a central position in receptor regulation. Hypophysectomy has been found to reduce Prl receptors, and this effect is partially prevented by a pituitary gland implanted under the kidney capsule (75). An intact pituitary is also a prerequisite for the receptor-inducing activity of estrogen (76). The nature of the pituitary factor that maintains the Prl receptor concentration in female rat liver has been the subject of several investigations. Based on experiments using Prl-secreting tumors and injections of heterologous hormones into hypophysectomized rats, it has been postulated that the Prl receptor-inducing factor is identical to Prl (75, 77, 78). The stimulation of the Prl receptor concentration by Prl itself would thus be an exception from the general rule of "down regulation" of receptors. However, conflicting data challenging the receptor-stimulating role of Prl have been reported, e.g. induction of Prl receptors following transplantation of GH-secreting pituitary tumors (79), absence of effect following injection of Prl into hypophysectomized animals (76), and inability of CB-154, a serum Prl-decreasing drug, to influence hepatic Prl receptor concentration (71).

Also, other functions of the liver besides the Prl receptor level are sexually differentiated, i.e. metabolism of drugs and hormones (for review see 80). In general, male rat livers display a higher hydroxylating activity than female livers. The maintenance of a female enzyme pattern is due to the secretion of a pituitary "feminizing factor" (see above). The sexually differentiated Prl receptor concentration and steroid and drug metabolism are regulated in a quite similar way and it is not unlikely that a similar or an identical pituitary factor is involved (81).

Hepatic Estrogen Receptors

Target tissues for estrogens contain a soluble protein which binds estrogen with a high affinity and a low capacity (82). This protein, the estrogen receptor, has been purified from calf uterus (83). It is generally assumed that following binding of estrogen to its receptor, the hormone-receptor complex diffuses into the cell nuclei where specific effects are exerted on transcriptional processes (2). Besides the well-known estrogen target tissues, human and rat liver cytosol has also been reported to contain estrogen receptors (84, 85). The receptor has mainly been studied in the female rat liver, but more recent publications have reported its occurrence also in the male rat liver (86). In mammals, estrogen administration results in several changes, which may be attributable to the presence of a hepatic estrogen receptor. Estrogen causes changes in plasma lipoproteins and lipids (87), increased concentration of clotting factors (88), and increased production of renin substrate (89). These actions of estrogen have led to the suggestion that the hepatic estrogen receptor might mediate side effects of estrogen therapy (90). In the rat, the level of hepatic estrogen receptors is increased after puberty, when estrogen-induced renin substrate production is initiated (89). Also, the estrogen receptor in the liver is under endocrine control. It has been reported that hypophysectomy reduces the receptor level and that ovine Prl may partially induce the Prl receptor concentration (90).

CENTRAL CONTROL OF PROLACTIN AND ESTROGEN RECEPTORS IN RAT LIVER

Hepatic Prl receptors constitute an example of a sexually differentiated liver function. As outlined above, the presence of Prl receptors in female rat liver depends on the pituitary secretion of a hormonal factor. In addition, the gonadal steroids influence the level of Prl receptors. Also, the hepatic estrogen receptor in the rat seems to be regulated by pituitary hormones.

The present review addresses the following problems:

1. Mechanisms involved in induction of Prl and estrogen receptors in rat liver and nature of the pituitary hormone responsible for receptor induction.
2. Mechanisms of hypothalamic control of hepatic Prl receptors, including identification of specific brain areas and possible neuroendocrine mediators responsible for regulation of Prl receptors.
3. The mechanism of action of estrogens and androgens in control of Prl receptors.

The Pituitary Factor Responsible for Regulation of Liver Prolactin Receptor Levels

Studies in our own laboratory (91) have confirmed previous investigations carried out by other workers (72, 75) showing that hypophysectomy of female rats reduces liver Prl receptors to a level normally found in male rats. Furthermore, a pituitary implanted under the kidney capsule could partially restore receptor levels in hypophysectomized female rats. Kinetic data calculated according to Scatchard (92) indicated that the receptor induction was due to an increase in the number of binding sites rather than to changes in receptor affinity. The results of renal pituitary implantation indicate that a pituitary hormone spontaneously released from the pituitary is responsible for induction of hepatic Prl receptors. The hormone predominantly secreted from renal pituitary transplants is Prl (93), but GH and the postulated "feminizing factor" with actions on hepatic steroid metabolism are also secreted (94, 95). Attempts have been made in our laboratory to characterize further the receptor-inducing hormone. The situation achieved by implantation of pituitaries under the kidney capsule, i.e. a continuous and long-term hormone secretion, was accomplished by using osmotic minipumps. Placed subcutaneously, this device slowly releases its content due to osmotic pressure; the minipump is emptied after one week (96).

hGH has dual functions in the rat, being both growth-promoting (97) and lactogenic (98, 99). For this reason, hGH was used to simulate the biological activities of rGH and rPrl in order to see whether these hormones were related to a receptor-inducing hormone.

When hGH was infused into hypophysectomized rats for one week, the hepatic Prl receptors were induced to a female level. This result indicates that hGH mimics the activity of a postulated pituitary factor in control of liver Prl receptor concentration. The effect of hGH in intact male rats was studied as a function of time, and it was found that induction of hepatic Prl receptors to a feminine level was complete following 4–6 days of hormone administration. Also, when male rats were given varying doses of hGH, a graded response was seen. Various endocrine organs have been reported to participate in liver Prl receptor regulation (72, 73, 100). Therefore, the effect of hGH was investigated in animals where gonads, thyroid, or adrenals were removed; in all experimental animals the receptor-inducing effect of hGH was unchanged. These results indicate that the ablated organs are not mediators of the receptor-inducing effect of hGH (101).

A dose of 5 μg/h of hGH, which causes a complete induction of hepatic Prl receptors to a female level, increased serum levels of hGH by approximately 10 ng/ml, indicating that low serum levels of hGH may cause effects on the rat liver. Human chorionic somatomammotropin (hCS) did not

induce Prl receptors (using an infusion rate of 5 μg/h) (102); this may indicate a specificity of the hGH induction of Prl receptors, as hGH and hCS are closely related proteins having more than 80% of their 191 amino acids in common (103, 104). Using the technique of hormone administration by way of osmotic minipumps, attempts were made to identify the endogenous rat hormone responsible for hepatic Prl receptor induction (101). When administered at a dose of 10 μg/h, rPrl did not affect hepatic Prl receptors, whereas rGH caused an increase in the Prl receptor level to 37% of the female control level. The receptor induction was accompanied by an increase in the serum levels of rGH. Administration of a somewhat more pure preparation of rGH (NIAMDD-I-4) caused a more pronounced effect on hepatic Prl receptors (to 75% of the female control value). Furthermore, the increase in serum GH also appeared higher in this experiment than following administration of the less pure rGH preparation.

When rGH was given to hypophysectomized rats at an infusion rate of 10 μg/h, serum levels of GH became 120 ng/ml as measured using RIA, which may be compared to serum GH in normal rats which vary between 20 and >500 ng/ml. The observation that rGH can induce hepatic Prl receptors may indicate that GH is similar or identical to the pituitary Prl receptor-inducing factor. At the infusion rate used, rPrl was not effective in inducing liver Prl receptors in either male or female hypophysectomized rats. This apparently could not be explained by rPrl blocking the hepatic Prl receptors, since in vitro dissociation of bound, endogenous Prl (using 3 M $MgCl_2$) followed by Prl receptor assay did not reveal any higher binding in rPrl treated animals compared to controls (101). In contrast to rPrl, heterologous Prl such as oPrl partially induced hepatic Prl receptors, an observation previously made by other workers (78). The differences in biological effects between Prl preparations derived from different species might be explained by structural differences between these Prl preparations. The possibility that rPrl was inefficient because of degradation of the hormone inside the minipump appears less likely, as treatment of rPrl under similar conditions to which it was exposed in the minipumps did not reduce its capacity to bind to the hepatic Prl receptor in vitro (101). In conclusion, rGH may cause induction of liver Prl receptors, whereas rPrl is less efficient in this regard.

Control of Estrogen Receptor Levels in Rat Liver

Using isoelectric focusing in polyacrylamide gel for quantitation of the estrogen receptor in rat liver, we have shown that the receptor is present at similar concentrations in liver from adult male and female rats (105). This agrees with results obtained using other techniques for estrogen receptor measurement (106). It was also shown that hypophysectomy reduces

estrogen receptor levels in the liver (105), confirming results of other investigators (70). Since it has been suggested that Prl is involved in the control of hepatic estrogen receptor levels (70), hypophysectomized rats were given renal pituitary implants in order to increase serum levels of Prl. It was found that the hepatic estrogen receptors were induced to 37% of the level of intact control rats, suggesting that other factors besides Prl participate in control of estrogen receptors in rat liver (105). In further experiments (107), adrenalectomy was found to reduce hepatic estrogen receptor levels. In view of this, hypophysectomized female rats with renal pituitary implants were also given glucocorticoid substitution; this combined treatment proved to be more efficient in inducing hepatic Prl receptors than pituitary transplants alone. In order to shed further light on the pituitary factor inducing liver estrogen receptors, hGH was infused in hypophysectomized rats using osmotic minipumps. Human GH was found to cause an increase in the hepatic estrogen receptor level, although the control level was not reached. However, a complete induction of hepatic estrogen receptors was seen when infusion of hGH was combined with daily injections of dexamethasone. These data suggest that a pituitary hormone with a similar function to hGH in combination with glucocorticoid is responsible for the induction of estrogen receptors in rat liver. Attempts were made to distinguish between the growth promoting and lactogenic properties of hGH with regard to its effects on liver estrogen receptors. Therefore, oPrl or oGH as well as a combination of these two hormones were administered to hypophysectomized rats. In all three experiments, the estrogen receptor level was induced. However, the effects were not as marked as when using hGH. It was concluded that the liver estrogen receptors are under multihormonal control. Possibly Prl, GH, and glucocorticoids may all be involved. It was, however, recognized that conclusions drawn from experiments where heterologous hormones were injected into the rat might be misleading (107).

A Comparison Between the Regulation of Prolactin and Estrogen Receptors in Rat Liver

From the data presented above, it is clear that pituitary factors, similar to hGH in function, induce both Prl and estrogen receptors in rat liver. Since the Prl receptor is sexually differentiated whereas the estrogen receptor is not, it was argued that the hormonal mechanisms inducing the receptors must be different in some way. Other workers did not observe any induction of the hepatic Prl receptor following injection of hGH into male rats (108). Furthermore, the secretory pattern of GH is sexually differentiated in the rat (49), and it was felt that possibly the mode of hGH administration was of importance for the type of biological action of the hormone. In order to investigate this possibility, the same daily dose of hGH was administered

to hypophysectomized rats at different intervals, ranging from injections every 12 h to continuous infusion (109). The most marked effect on hepatic Prl receptors was observed following continuous infusion of hGH, whereas no effect was seen following injection of hGH every 12 h. On the other hand, hGH was seen to exert the same effect on body weight (an increase) and tibia epiphyseal zones (a widening) irrespective of the mode of administration. It was therefore concluded that different effects of hGH were seen depending on the mode of administration. When continuous and infrequent administration of hGH were compared with regard to effect on the hepatic estrogen receptor level, only the continuous mode of administration was efficient in inducing the receptor. Thus, a similar requirement for continuous hormone administration was found for both estrogen and Prl receptors. rGH and rPrl were tested for their capacity to influence Prl and estrogen receptors in hypophysectomized rats. The Prl receptors were most markedly induced by rGH, confirming previous results (see previous section). Also, the estrogen receptor was partially restored following treatment with rGH, whereas no effect was seen when rPrl was infused. It therefore seems as if rGH influences both sexually differentiated functions of the liver (Prl receptors) as well as sexually nondifferentiated liver functions (estrogen receptor).

Hypothalamic Control of Prolactin Receptor Levels

The pituitary hormone which feminizes hepatic steroid metabolism has been shown to be under control from the hypothalamus (110). Experiments were undertaken to investigate whether a similar regulatory mechanism could be demonstrated in the case of the hepatic Prl receptor. The study also aimed at localizing discrete brain areas involved in this control and to investigate neuroendocrine events responsible for receptor induction. Deafferentation, i.e. severing nerve fibers entering the hypothalamus, was achieved by using specially designed knives. When male rats were deafferentated at the suprachiasmatic level (anterior deafferentation), an increase in the hepatic Prl receptor concentration was seen (91). This increase, which led to a female level of Prl receptors, was observed 3–4 days following the operation. This finding suggests that by disrupting neural pathways in the male hypothalamus it is possible to initiate secretion from the pituitary of a receptor-inducing hormone. Obviously, secretion of the receptor-inducing pituitary factor is normally inhibited by the male hypothalamus.

Ovariectomy of female rats led to a certain reduction in the concentration of hepatic Prl receptors. This reduction was completely reversed by deafferentation of ovariectomized female rats, which indicates that estrogen is not absolutely required for complete induction of hepatic Prl receptors (91).

Serum levels of Prl were not elevated in deafferentated rats, a finding that agrees with observations by other workers; probably the inhibitory center for Prl release remains intact in animals subjected to anterior deafferentation (111). Furthermore, transections rostral to the suprachiasmatic nucleus had no effect on liver Prl receptors in male rats (91). Taken together with the results referred to above, these findings might be interpreted to mean that an inhibitory center for pituitary release of Prl receptor-inducing hormone is located between the level of the rostral transection and the level of the anterior deafferentation, or that inhibitory nerve fibers from the lateral part of the brain pass through the region between the two levels of transection. The latter possibility is supported by the finding that large lesions in the amygdaloid complex in male rats cause a slight increase of hepatic Prl receptors (112). However, a more pronounced receptor induction was seen when small lesions comprising the area between the levels of the rostral transection and the anterior deafferentation were performed. These small anterior hypothalamic periventricular lesions destroyed tissue surrounding the third ventricle. The marked effects of these lesions on hepatic Prl receptor levels suggest that this region might be identical to the postulated hypothalamic inhibitory center which, in turn, might be influenced by extrahypothalamic (amygdaloid) structures. Alternatively, the periventricular area represents "a final common pathway" for inhibitory signals derived from extrahypothalamic sources (112). Clusters of somatostatin-containing nerve cells have been localized in the periventricular area and lesions destroying these cells reduce the somatostatin found in the median eminence (113, 114). Furthermore, transections similar to the anterior deafferentations mentioned above reduce the content of somatostatin in the median eminence and also alter the secretory pattern of GH (115).

In view of the findings concerning the hypothalamo-pituitary regulation of hepatic Prl receptors (91), one may speculate that somatostatin is the neuroendocrine regulator of these receptors. Administration to male rats of an antiserum against somatostatin resulted in an increase in the level of hepatic Prl receptors (112). It was also found that lesions in the periventricular area resulted in reduced immunofluorescence of somatostatin cell bodies in the periventricular area as well as reduction of somatostatin fibres in the median eminence, and that these lesions resulted in an induction of hepatic Prl receptors. Somatostatin-like immunoreactivity in the median eminence was quantified by radioimmunoassay. Following anterior hypothalamic periventricular lesions, somatostatin levels were decreased to 2–10% of control values (112). When somatostatin was injected into female rats, the Prl receptors were reduced to approximately 60% of control level. In order to obtain this effect of somatostatin, high and frequent doses of somatostatin had to be injected. The data indicate that somatostatin could

play an important role in the regulation of Prl receptors in the liver. Furthermore, the sexually differentiated steroid metabolizing enzymes (metabolism of 4-androstene-3,17-dione) appeared to be regulated in a similar manner (112). This finding supports the contention that the control of Prl receptors may be regarded as one of many sex-differentiated liver functions regulated by the pituitary in a similar fashion.

The concept of somatostatin as a central effector of sex differentiation of the liver should be regarded with some caution as brain lesions and antibody treatment are likely to interfere with several neuroendocrine systems.

The Role of Estrogen and Androgen in Control of Hepatic Prolactin Receptor Levels

As stated in the introduction, estrogens increase hepatic Prl receptors. It has been suggested that the effect of estrogens might be at the hypothalamo-pituitary level, as estrogens have no effect in hypophysectomized animals (76). However, a direct effect of estrogens on the liver has also been suggested (71). The possibility of an effect of estrogens on the hypothalmo-pituitary unit was studied by placing estrogen-containing implants at various intracranial locations. It was found that when estrogen was placed in the pituitary region of male rats, hepatic Prl receptors were increased to a female level (116). The receptor-inducing effect of estrogen implants was more pronounced when the implants were placed in the pituitary region than when they were placed in the anterior hypothalamic area. The experiments indicated that estrogen acts at the hypothalamo-pituitary level, possibly directly on the pituitary (116). It is difficult to determine the exact site of estrogen action as the brain area affected by estrogen diffusion is unknown. A direct effect of the estrogen on the pituitary is supported by the finding that estrogen amplifies the inductive capacity of a renal pituitary implant (75).

Androgens reduce the level of hepatic Prl receptors. It has been suggested that the target for this action of androgen is the liver (74). Following anterior hypothalamic deafferentation of female rats, this effect of androgen was no longer seen (116). Since the effect of androgen on hepatic Prl receptors was abolished following destruction of certain neural pathways in the brain, it is quite possible that androgens act at the hypothalamic level, most likely in the rostral hypothalamus or adjacent areas (116).

In summary, it is possible that both androgens and estrogens exert their effects via the pituitary Prl receptor-inducing factor, and that the primary site of action of sex steroids is the pituitary region, in the case of estrogens, and the anterior hypothalamus in the case of androgens.

CONCLUSION

This review presents data indicating that hepatic Prl receptors in the rat are regulated by changes in number of binding sites rather than by alteration of binding affinity. According to the general concept, induction of hormonal receptors most commonly is the result of synthesis of new receptor protein (117). Such a mechanism is also the likely explanation for the observed induction of hepatic Prl receptors. Furthermore, the relatively long time required for induction of hepatic Prl receptors suggests involvement of complex biochemical events.

The concept that Prl induces its own receptor does not seem to be based on a solid experimental basis; it would rather seem as if GH is the hormone responsible for Prl receptor induction. A role of Prl may, however, not be completely ruled out until the effects of Prl have been tested using varying concentrations of the hormone. Neither should one disregard the possibility of a combined effect of GH and Prl in inducing hepatic Prl receptors in the rat. Therefore, additional studies are necessary before a role of GH as a physiological regulator of sexual differentiation of liver functions may be finally stated. Several results indicate that GH has an important role in induction of hepatic Prl receptors. Furthermore, recent studies also indicate a role of GH in regulating the sexually differentiated steroid and drug metabolism (118, 119).

The way in which GH exerts its control of the sexually differentiated liver functions is obscure. It has been reported that GH consists of several subforms or isohormones (15–18, 120, 121). Possibly the GH isohormone pattern is sexually differentiated; it cannot be excluded that a female-specific "feminizing factor" or Prl receptor-inducing factor exists and that this factor is identical to one of the GH isohormones. Another important phenomenon that might be of significance in this respect is the sexually differentiated GH secretory rhythm. It has been suggested that sex differences in somatic growth could partially be explained by sex differences in GH secretory patterns (122). Possibly, the secretory rhythm of GH may also be of importance for other actions of GH, such as various liver functions. Experimentally, it is possible to change the secretory rhythm of GH in rats by performing anterior hypothalamic deafferentation or by inducing lesions in the anterior hypothalamus (123). The changes in secretion of GH are believed to be due to disruption of the inhibitory hypothalamic control of GH secretion from the pituitary, since somatostatin levels are reduced in the median eminence (115). Sex differences in the secretory rhythm of GH have been postulated to be due to sex differences with regard to the function of the anterior hypothalamic GH inhibitory area (124). The results pre-

sented in this review may be a functional correlate to these changes in hormone secretion, since anterior hypothalamic deafferentation induces hepatic Prl receptors and since periventricular lesions, besides reducing the concentration of somatostatin in the median eminence, also induce Prl receptors. In view of these results, one may speculate that there exists a center in the anterior hypothalamus that is responsible for a sexually differentiated secretion of somatostatin leading to a sex-specific GH rhythm or to a sex-specific GH isohormone pattern, which in turn leads to a sexually differentiated rat liver. The gonadal steroids influence the hepatic Prl receptor concentration via the hypothalamo-pituitary-liver axis. The receptor-stimulating effect of estrogen could possibly be exerted directly at the pituitary level. The receptor-decreasing effect of androgen could be exerted in the anterior hypothalamus.

It thus seems as if gonadal steroids influence the concentration of hepatic Prl receptors indirectly, which suggests that androgens and estrogens regulate secretion of a pituitary receptor-inducing hormone. In this context, it is of interest to note that sex steroids are known to modulate pituitary Prl and GH secretion. Estrogens increase serum Prl levels, and one target tissue for this action of estrogens is the pituitary gland (125, 126). Also, androgens seem to stimulate serum Prl levels (127). This effect is, however, not seen when using 5α-dihydrotestosterone (128). There is no general agreement concerning the effects of steroid hormones on GH synthesis and secretion or on the steroid target sites involved (129, 130). Estrogens may lower the content of pituitary GH and increase the concentration of GH in blood (125, 129). Androgens might decrease the secretion of GH or be without effect (129, 131). The effect of sex steroids on the secretory rhythm of GH or on the secretion of GH isohormones is not clear. Studies on the hormonal regulation of the secretory rhythm of GH may lead to a further understanding of the nature of the Prl receptor-inducing hormone.

The hepatic estrogen receptor was found to be present at similar concentrations in both sexes. Induction of this receptor was achieved by infusing hGH; the response was amplified by combining hGH with glucocorticoid treatment. Such multihormonal regulation has also been shown for other systems, e.g. the production of α_2 urinary globulin by rat liver (132). A partial induction of estrogen receptors was observed following infusion of rGH. Interestingly, estrogen receptors appear in rat liver at the time of puberty (89); at this time GH secretion is also altered (49). The regulation of the hepatic estrogen receptor showed certain similarities with the regulation of the hepatic Prl receptor with regard to the involvement of pituitary control: both receptors were stimulated by GH if continuously infused. What remains to be explained is how GH action on rat liver results in sexually differentiated Prl receptor concentrations and in sexually non-

differentiated estrogen receptor levels. Further detailed studies on the endocrine control of rat liver with simultaneous measurements of Prl and estrogen receptors may help to clarify the mechanisms behind this dual function of GH.

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