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Role of the Hypothalamo-Pituitary-Liver Axis in Sex Differences in Susceptibility of the Liver to Toxic Agents

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At birth testicular androgens irreversibly program brain centers involved in hypothalamo-pituitary control of hepatic sex-dependent steroid and drug metabolism. This imprinting process results in activation of a hypothalamic "feminostatin"—a secreting center that is turned on just before puberty. Feminostatin inhibits pituitary secretion of "feminizing factor," a pituitary hormone that feminizes the basal type of metabolism characterizing the liver of hypophysectomized and gonadectomized rats. Consequently, female rats that are devoid of feminostatin will secrete feminizing factor from the pituitary, leading to a feminine type of hepatic metabolism. Male rats have an active feminostatin-secreting center, and inhibition of pituitary feminizing factor release results in an autonomous type of liver metabolism. Female rats show a relative androgen unresponsiveness and seem incapable of releasing feminostatin after treatment with natural androgens, possibly because of more efficient metabolism (breakdown) of androgen in the female than in the male rat brain.

Frontal deafferentation at the retrochiasmatic and suprachiasmatic level resulted in a complete "feminization" of hepatic steroid metabolism in male rats. Such an effect was also seen when lesions involving mainly the anterior periventricular hypothalamic area and the suprachiasmatic nucleus were performed in male rats. No effects were seen in analogous lesions in female rats in any of the cases studied. These findings suggest that a region including the anterior hypothalamic periventricular area, the suprachiasmatic nucleus and adjacent areas is involved in the control of hepatic steroid metabolism. It is postulated that the neuronal cell bodies that produce feminostatin have their origins in this area of the hypothalamus or may send axons through this area to the basal hypothalamus and thus directly or indirectly influence the anterior pituitary gland.

Regulation by the central nervous system of a "lactogenic" (prolactin, Prl) receptor, present in the female rat liver, was also studied. This receptor is present in very low concentration or absent in the male rat. Anterior hypothalamic deafferentation at the retrochiasmatic level in male rats increased the hepatic Prl receptor concentration to the female level 3-4 days following the operation. A transection rostral to the suprachiasmatic nucleus had no effect on the concentration of Prl receptors in male animals. Our results demonstrate that the number of Prl receptors is regulated by the hypothalamo-pituitary system. The receptor-inducing pituitary factor might be related to the feminizing factor. Experiments were carried out to elucidate the nature of the Prl receptor-inducing pituitary factor.

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Human growth hormone (hGH) continuously administered was shown to induce Prl receptors in livers from male and female hypophysectomized-gonadectomized rats. The prolactin receptors were increased to a level found in control female rat livers. This inductive effect of hGH was also seen in adrenalectomized and thyroidectomized male rats. The induced Prl receptors in male rats had similar characteristics as hepatic Prl receptors in female rats.

Also the endogenous rat hormones, rPrl and rGH, were administered in minipumps. In the concentration used (10 μg/μl), rPrl had no effect whereas rGH increased Prl receptor levels to approximately 37% of the female control level. These results indicate that GH or a peptide related to GH may be involved in the regulation of hepatic Prl receptors.

The hypothalamo-pituitary-liver axis represents a new concept in endocrine regulation of drug toxicity. The male rat liver has been shown to be more susceptible than the female rat liver to the hepatocarcinogenic action of certain drugs, and it is conceivable that sex differences in the metabolic activation of the drugs in the liver may explain the greater sensitivity of male rats to chemically induced hepatocellular carcinoma. Similar sex differences in liver cancer incidence have been reported in the human.

Introduction

Primary liver cancer has been reported to be more frequent in men than in women (*1-4*) and a greater male susceptibility to the induction of liver cancer by carcinogens such as *N*-2-fluorenylacetamide has also been found in several rat strains (*5-7*). Removal of the testes decreased the tumor incidence in the experimental animals (*8, 9*) and substitution with androgen restored the responsiveness (*8*). Long-term treatment with androgenic steroids has also been claimed to increase the risk for development of hepatocellular carcinoma in humans (*10*). In rats, testosterone was reported to act indirectly via endocrine tissue(s) to increase chemical hepatocarcinogenesis (*11*). Recently, Toh found that bilateral electrolytic lesions placed in the median eminence area of the hypothalamus in adult male rats had an inhibitory effect on liver tumor induction by *N*-2-fluorenylacetamide (*12*). This experiment indicated a role of the hypothalamus in determining the susceptibility of the liver to toxic agents.

Since several years we have been involved in studies on mechanisms involved in regulation of sex differences in hepatic metabolism of steroids and drugs (*13*). Based on our findings, we have suggested the existence of a novel endocrine control system, the hypothalamo-pituitary-liver axis, of major importance in regulation of hepatic metabolism. It is apparent to us that the results on liver toxicity described above may well be understood in view of this new concept of control of liver function and therefore the present paper will review some aspects of hypothalamo-pituitary regulation of hepatic metabolism.

Central Control of Hepatic Steroid and Drug Metabolism

In the early 1950's, Hübener and Amelung (*14*) showed a sex-related difference of hepatic corticosteroid metabolism in the rat. This finding was substantially confirmed by subsequent reports using corticosteroids (*15-17*), androgens (*16-18*), and estrogens (*18*) as substrates. Male animals show a higher hydroxylating activity than females (*17, 18*), whereas females exhibit a higher 5α-reductase activity (*16*). Somewhat later, Yates et al. (*16*) published the first study indicating that androgens were involved in the control of steroid metabolism in rat liver. It was found that castration increased and testosterone treatment decreased the 5α-reductase activity. This finding was confirmed and extended by Gustafsson and Stenberg (*19-22*), who showed the dependence of the adult male pattern of metabolism on the presence of neonatal androgen (imprinting). Although dependent on neonatal androgen exposure, no sex-related differences in steroid metabolism were observed until 30 days of age (*23-27*). Sex-related differences in metabolism and the androgen-dependence of these differences have also been observed for the metabolism of various drugs and xenobiotics including ethylmorphine (*28*), aniline (*29*), *p*-nitroanisole (*30*), and hexobarbitone (*31*). The absence of any sex-specific androgen receptor in the liver (*32*) coupled to the fact that male animals are more sensitive to androgen action as regards hepatic steroid metabolism in the adult period (*22, 33*) seems to indicate an indirect action of androgen on hepatic steroid metabolism.

A possible explanation of the indirect action of androgens on hepatic steroid metabolism was put forward in 1974, when Denef (*34*) and Gustafsson and Stenberg (*35*) published the effects of hypophysectomy on hepatic steroid metabolism. Both groups showed that hypophysectomy of female animals resulted in a male pattern of metabolism, indicating that the female pattern of metabolism is dependent on the presence of an intact pituitary. It is well known that the hypothalamus in the rat is sex-differentiated with regard to control of pituitary secretion (*36*) and thus it is likely that the

sex-related difference in hepatic steroid metabolism observed is secondary to the sexual differentiation of the brain at birth and that androgens have their action via the hypothalamo-pituitary axis rather than directly on the liver. The pituitary dependence of hepatic metabolism is not confined to steroid metabolism although both androgen (*34*, *35*) and corticosteroid (*37*) metabolism are involved. Kim et al. (*38*) have shown that protein demethylase II activity in the rat liver is pituitary-dependent and there is evidence to support the pituitary dependence of drug and xenobiotic metabolism, including that of ethylmorphine (*39*, *40*). The basal hepatic lipolysis (*41*) and synthesis of plasma proteins (*42*) have also been shown to be controlled, at least partially, by the pituitary gland. Kramer et al. (*39*) have shown that the effect of testosterone on ethylmorphine metabolism is not manifested in the absence of the pituitary gland. A similar observation was seen in the study of Gustafsson and Stenberg (*35*) where neither testosterone nor 17β-estradiol had any major effects on hepatic steroid metabolism in hypophysectomized rats. It thus seems likely that the effects of the sex steroids on hepatic metabolism are indirect and are effectuated via the hypothalamo-pituitary axis.

Implantation of a pituitary gland under the kidney capsule of a hypophysectomized male or female rat resulted in feminization of the host's hepatic steroid metabolism (*34*, *43*). We also showed that different time periods were required for the changes in different enzyme activities (*44*), emphasizing the importance of the sensitivity of the respective enzyme system to the controlling factor(s). This would seem to indicate that the ectopic pituitary gland secretes a factor into the general circulation that is normally only secreted by the female pituitary gland *in situ*. The ectopic pituitary tissue is known to secrete prolactin and growth hormone and small amounts of the other anterior pituitary hormones (*45-48*). In our experiments only prolactin and growth hormone could be measured in the serum of implanted animals (*44*). The hypophyseal factor responsible for the maintenance of the female-type of hepatic metabolism is, as yet, an open question.

A number of reports have been published showing effects of various purified pituitary hormones or combinations of hormones on drug and steroid metabolism in the rat. Gustafsson and Stenberg (*49*, *50*) have shown an effect of FSH but no effect of LH or prolactin on hepatic steroid metabolism when 4-androstene-3,17-dione is used as substrate. These results are in agreement with those of Colby et al. (*37*), where corticosterone was used as substrate, and gain further confirmation from the study of Lax et al. (*45*), where the effect of prolactin on reductive metabolism was examined. In the report of Colby et al. (*37*), a combination of GH and ACTH decreased reductive metabolism (an effect similar to hypophysectomy), while Lax et al. (*51*) found that prolactin had this effect. A later report by Kramer and Colby (*52*) indicated that GH alone increased reductive metabolism of steroids (an effect dramatically opposite to that of GH combined with ACTH). In the field of drug metabolism, ACTH and GH seem to be the most active. GH alone was thought to reduce the hepatic metabolism of ethylmorphine (*52*, *53*) and aminopyrine (*53*), whereas combinations of ACTH and GH have been shown to increase the carcinogenicity of *N*-hydroxy-*N*-2-fluorenylacetamide (*54*, *55*) (thereby indicating a decrease in dehydroxylation and deacetylation of the carcinogen by the liver). Kramer et al. (*56*) have reported that both LH and FSH, when given to castrated male and female rats, increased ethylmorphine demethylase activity in the liver.

None of the above mentioned effects are, however, as marked as that seen after implantation of a pituitary gland under the kidney capsule. These data would indicate that the control of hepatic steroid and xenobiotic metabolism is mediated via an, as yet, unidentified pituitary factor (or possibly an unidentified combination of factors)—we have labelled this factor "feminizing factor" or "feminotropin." The purification of this factor is underway in our laboratory.

Studies by our group (*57*, *58*) on the ontogenesis of the pituitary control of hepatic steroid metabolism have indicated that the liver and pituitary are not per se sex differentiated with regard to their response to and production of "feminizing factor," respectively. Thus, the masculinized ("imprinted") control center must lie elsewhere.

The possibility that hepatic steroid metabolism is under indirect hypothalamic control was first indicated by our above-mentioned finding (*59*) that complete electrothermal destruction of the hypothalamus in male rats resulted in "feminization" of the steroid metabolism in the liver of the operated animals. This indicated for the first time that, in the male rat, release of the factor(s) maintaining a female-type steroid metabolism in female animals was inhibited by the hypothalamus. This inhibitory control is similar to that of prolactin, the release of which is inhibited by prolactin release inhibiting factor (PIF) (*60*).

The site of the sexual differentiation in the hypothalamus appears to lie predominantly in the preoptic area where, in fact, anatomical differences in nerve structure can be found. Greenough et al.

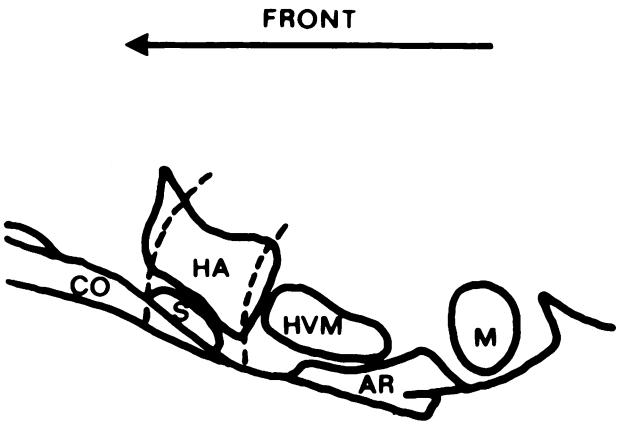

FIGURE 1. Diagrammatic representation of the level of deafferentation used. Deafferentation at the retrochiasmatic level caused complete "feminization" of hepatic steroid metabolism: (CO) optic chiasma, (HA) anterior hypothalamic nucleus, (S) suprachiasmatic nucleus, (HVM) ventromedial nucleus, (AR) arcuate nucleus, (M) mamillary body.

(*61*), on studying the hamster preoptic area, found different patterns of dendrite density corresponding to different afferent inputs to the dorsomedial preoptic area. In addition, many reports have been published on the sex differences in hypothalamic structure and their relation to neonatal androgen exposure (*62-65*).

In order to ascertain more exactly the area of the hypothalamus that controls the secretion of feminizing factor, a series of experiments was performed by our group (*66*) whereby the effects of various brain lesions on hepatic steroid metabolism were investigated. Two distinct types of lesions were performed: one, anterior hypothalamic deafferentation and two, discrete lesions in the rostral parts of the hypothalamus. One week after the lesioning, the animals were killed and their hepatic steroid metabolism investigated. The effects of the lesions on steroid metabolism were correlated to the changes in secretion of pituitary hormones.

Hypothalamic deafferentation about the level of A5660μ [according to the atlas of Jacobovitz and Palkovits (*94*)] (posterior to the preoptic area and thus separating the rostral parts of the hypothalamus from the medial basal hypothalamus including the median eminence) (Fig. 1) caused complete "feminization" of hepatic steroid metabolism in the operated male animals while having little effect on the female animals (*66*). These results indicate that the center of control lies anterior to the level of A5660μ, possibly in the suprachiasmatic nucleus or in the surrounding periventricular area. This finding is in contrast to the proposed regulatory site for prolactin secretion which lies within the boundaries of the deafferentation. In order to further investigate the possible involvement of the hypothalamic areas, a number of discrete lesions were placed in various positions in this area and their effects on hepatic steroid metabolism studied. A comparatively large midline lesion placed in the periventricular region and stretching between levels A7190μ and A5660μ (i.e., roughly located anterior to the level of the deafferentation) caused complete feminization of steroid metabolism in the livers of operated male animals while having only small effects on female animals. However, lesions in the midline but occupying only the more rostral aspects of the lesion mentioned above (stretching from levels A7890μ to A6670μ) were without effect indicating that the essential areas involved in the control of the secretion of feminizing factor lie caudally to the level A6670μ; i.e., approximately extending from the beginning of the suprachiasmatic nucleus in a caudal direction.

Bilateral lesions essentially involving the nuclei interstitialis striae terminalis but also extending medially including the anterior commissure caused a moderate degree of feminization indicating that higher centers may be involved in the regulation of the secretion of feminizing factor. Small bilateral lesions in the lateral preoptic area were without effect on steroid metabolism in the livers of the lesioned rats.

The question may now be asked whether the effects of androgens and estrogens on the liver may be mediated via the hypothalamus. Much work has been done on the presence of receptors for sex steroids in the brain particular as regards the hypothalamus. We have shown the existence of specific androgen receptors in several brain regions including the hypothalamus, cortex and pituitary gland (*67*). In the same study we found that there appears to be a sexual dimorphism of androgen metabolism in rat brain (*67*). Testosterone was metabolized predominantly to androstenedione in the male but to 5α-androstane-3α(or 3β), 17β-diol, epitestosterone and dihydroepitestosterone in the female. The metabolism of testosterone was also much faster in female than male brains. This could explain the relative androgen unresponsiveness in the female.

With regard to the actions of estrogens, it is well accepted that specific estrogen receptors exist in the hypothalamus and that binding of estrogen to these receptors can cause biochemical and physiological changes in the receptor-containing brain areas. We have recently developed a highly sensitive and specific method for estradiol receptor quantitation, based on isoelectric focusing in polyacrylamide gel, and we have used this method

to study sexual differences in distribution of estradiol receptors in the brain (*68*). The highest concentrations of estradiol receptor were found in amygdala and anterior and posterior hypothalamus.

All of these data would seem to indicate that the hypothalamus (and pituitary gland) is capable of reacting to estrogens (in both sexes) and androgen (only in the male). It is thus likely that most, if not all, effects of sex steroids on hepatic steroid metabolism are indirect and mediated via the hypothalamo-pituitary system. Furthermore, it is the hypothalamus that is imprinted at birth by androgens exactly as has been demonstrated for the adult patterns of sexual behavior and release of gonadotropins.

Our proposed theory for the imprinting of hepatic steroid metabolism in the rat is shown in Figure 2. Imprinting occurs entirely in the hypothalamus where androgen in the neonatal period alters the anatomical, physiological and biochemical workings. This area, which we have labeled the "feminostatin" center, since we think it produces the feminizing factor release-inhibiting factor, is only active in the male. Androgen in the neonatal period is essential for activation of the center. In the normal adult male, the activated feminostatin center produces and releases feminostatin which, in turn, inhibits the release of feminizing factor from the pituitary gland. This leads to a masculine pattern of hepatic metabolism. In the female, the feminostatin center is inactive, and thus feminizing factor is released from the pituitary gland giving a feminine pattern of hepatic metabolism. Androgens are active in the male in the adult period by interaction with the androgen receptors in the brain while estrogens are active in both sexes via the estrogen receptors in the hypothalamus and pituitary gland. It is probable that the effects of the sex steroids are mediated via the feminostatin center, but a direct effect on the pituitary gland cannot be ruled out.

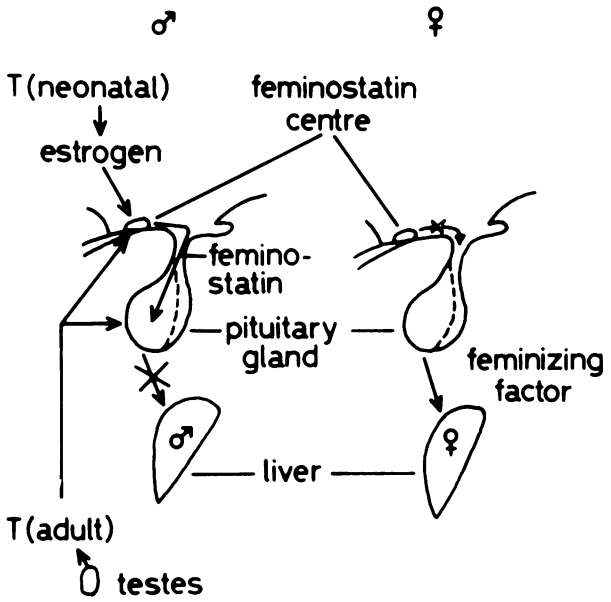

FIGURE 2. Proposed model for the control and imprinting of hepatic steroid metabolism in the rat. T denotes testosterone.

Central Control of Prolactin (Prl) Receptors in the Liver

During recent years, we have studied another sex-differentiated liver function quite extensively, namely the concentration of prolactin receptors. It has become more and more evident that also this system is controlled by the hypothalamo-pituitary axis and that, indeed, the feminizing factor or a similar factor may be responsible for the maintenance of prolactin receptors in the female rat liver. Some of our recent findings on regulation of hepatic prolactin receptors will be presented below.

In 1974, Friesen's group showed the existence of a receptor in rat liver that bound both ovine prolactin and human growth hormone (*69*, *70*). It was found that this receptor was present in much higher concentrations in female than male rats. These findings have been confirmed by other groups including our own (*71-74*).

The development of these receptors has been shown to follow that of the steroid-metabolizing enzymes rather closely. No sex differences were found in lactogenic receptor concentration in prepubertal rats, but between 20 and 40 days of age a marked increase in the receptor level in female rats occurred (there was no concomitant change in the male) (*70*). This should be compared to the development of, for example, the 5α-reductase (active on 4-androstene-3,17-dione) (*27*); this enzyme was found in low concentrations in prepubertal rats but developed rapidly in females around 30 days of age leading to the marked sex differences in activity noted in the adult period.

The level of "lactogenic" receptors in rat liver seemed to depend upon the level of androgen and estrogen. Ovariectomy reduced and castration increased the number of "lactogen" binding sites whereas replacement therapy with the appropriate steroid reversed the effect of the glandular ablation (*72*, *75*, *76*). Male animals injected with estrogens showed a marked increase in "lactogenic" binding while females treated with testosterone displayed lower binding than controls. This again is a very similar situation to that found for the enzymes

responsible for the metabolism of steroids and drugs in the rat liver, as discussed above.

Further work by Friesen et al. (*75*) and others (*71*) has shown that, even in this system, the pituitary gland has some role to play. Removal of the pituitary gland of female animals led to a reduction in the concentration of receptors to a male level whereas similar treatment of male animals was essentially without effect. Treatment of hypophysectomized males with estrogen was also without effect, indicating that the estrogen may be acting in an indirect fashion via the hypothalamo-pituitary axis or that both estrogen and a pituitary factor are necessary for the effect on the liver. It would thus seem from the above data that the effects of the sex steroids on hepatic "lactogenic" receptors are indirect and are mediated via the hypothalamo-pituitary axis.

What is the pituitary factor that maintains the high level of "lactogenic" binding sites in the female rat liver? Posner et al. (*76*, *77*) have shown that implantation of a pituitary gland under the kidney capsule of a hypophysectomized female rat could restore the original level of receptors, indicating that, in a similar manner to hepatic xenobiotic metabolism, the ectopic pituitary can secrete a factor that maintains the female-type liver. The time course of the effect of implantation of a pituitary gland on lactogenic receptor level follows very closely that of development of female steroid-metabolizing characteristics following pituitary implantation (*78*) but shows a marked lag period when compared to the outflow of prolactin from the transplant. It was suggested by Friesen that prolactin itself may be the inducer of lactogenic binding in rat liver. This assumption was based on experiments (*71*, *76*, *78*, *79*) whereby the effects of treatment with ovine prolactin, human growth hormone and ACTH on lactogenic receptor level were tested in hypophysectomized animals. Costlow et al. (*71*) found a restoration of lactogenic receptors in hypophysectomized female rats but only to 15% of the level found in control females, whereas Friesen et al. (*78*) showed an almost complete restoration of lactogenic binding in hypophysectomized female animals, by treating with a mixture of ovine prolactin and human growth hormone or ACTH and human growth hormone (human growth hormone itself was, however, a bad inducer, as was ACTH). In male animals (*79*), a mixture of human growth hormone and ACTH was the most effective in inducing lactogenic receptors. A growth hormone-producing tumor (GH_12C_1) was also shown to induce lactogenic binding in male rats, indicating an apparent role of growth hormone in the regulation of these receptors. Other pituitary tumors have been shown to induce lactogenic receptors including the prolactin-producing MtT/F_4 and MtT/W_5 tumors (*76*). It is interesting to note that both of these tumors have also been shown to feminize the rat liver with regard to steroid metabolism (*80*).

The hypothesis put forward that prolactin regulates the lactogenic receptors in rat liver no longer seems to hold true following the work of Posner (*76*), who showed that replacement therapy with pure ovine prolactin was without effect in hypophysectomized animals. Recently, treatment of hypophysectomized females bearing a pituitary graft with the dopaminergic agonist, CB-154 (discussed above), has been shown to be ineffective in eliminating the lactogenic receptor-inducing capacity of the implant although serum prolactin levels drop below the detectable limit (*75*).

In our own work (*74-81*), the prolactin-induced lactogenic receptor concept has been consistently challenged. We have also found no effect of CB-154 on hepatic receptor level either in normal or pituitary-transplanted animals. In addition, extensive work involving hypothalamic deafferentation has indicated no correlation between effect on serum prolactin level and "lactogenic" receptor level (see below).

Even the physiological ligand for the "lactogenic" receptor in rat liver is still in question. Friesen and his group have assumed that binding of ovine prolactin to rat liver is a measure of the binding of rat prolactin to the same receptor. This does not necessarily have to be the case, however, as indicated by the work of Posner (*76*) where both ovine prolactin and human growth hormone were shown to be about five times as effective as rat prolactin in displacing bound labelled ovine prolactin. Rat growth hormone did not displace ovine prolactin at all from the lactogenic receptor.

Ranke et al. (*82*, *83*), working with isolated hepatocytes, have shown that in this system rat prolactin was also 10 times less effective than ovine prolactin as a displacer and that rat growth hormone was also ineffective as a displacing agent. Other groups working with isolated cell systems have shown binding of ovine prolactin and human growth hormone (*84*, *85*) but no regulatory studies have yet been performed in this type of system.

To measure "lactogenic" receptor sites, we have used ^{125}I-labeled human Prl (Biodata, Coissons, Switzerland), iodinated by the chloramine-T method (*86*) to a specific radioactivity of approximately 100 Ci/mg, as a tracer. Incubations were carried out within 3 weeks of the iodination date. Some decline in apparent hormone binding, due to storage inactivation of the ligand, was observed during this period. Since no repurification of the iodinated

ligand was performed, pooled microsomal membrane fractions from female animals were included in the experiments as a control. Each incubation was carried out in 1.5 ml Eppendorf tubes (Eppendorf, West Germany) containing 0.5 ml of microsomal membrane suspension with 0.3-0.4 mg of protein as determined by the method of Lowry et al. (*87*). The tracer (approximately 30,000 cpm) was added in 0.1 ml of Tris buffer. Displacing agents were added in 0.1 ml of Tris buffer. Samples were slowly rotated for 22-24 hr at 4°C, after which the incubation was terminated by centrifugation for 5 min in a microfuge B centrifuge at 10,000g. The tip of the plastic Microfuge tube containing the pellet was removed and counted in a gamma scintillation counter. Routinely, each individual liver membrane fraction from a group of animals was assayed by a single point assay. For one animal in each group a Scatchard plot was constructed (*88*) after correcting for the protein concentration. As displacing agent, ovine Prl (NIH-P-S-12) was used, and when Scatchard analysis was performed, the displacing agent was used in a range of 0.001-1 μg per incubation. Nonspecific binding was defined as the radioactivity bound in the presence of 1 μg oPrl. Specific binding was calculated by subtracting nonspecific binding from total binding. All incubations were performed in triplicate or quadruplicate. Means and standard deviations were calculated for the various experimental groups and the degree of significance calculated using Student's *t*-test. The level of significance was set at $p < 0.05$.

Hypophysectomy caused a marked reduction in the number of hepatic hPrl receptors in female animals. The receptors could partially be restored by a pituitary implant under the kidney capsule. The low binding in hypophysectomized female animals resembled the binding found in males. Scatchard plots showed that hPrl receptor levels in the experimental group with pituitary implants were not completely restored to the control female level (binding capacity 76 as compared to 165 fmole of binding sites/mg protein) but that the K_D was in the same range (0.9 and 1.0 × $10^{-9}M$, respectively).

Experiments were also performed to study the effect of hypothalamic lesions on hepatic hPrl receptors. The effect of anterior hypothalamic deafferentation on hPrl receptors in liver was studied as a function of the length of the interval between operation and decapitation (Fig. 3). Male animals were deafferentated at the retrochiasmatic level at different times so that all animals in the experiment were killed at the same time. A group of sham-operated animals was included. It was found that suprachiasmatic deafferentation of male rats led to an increase of hepatic hPrl receptors to

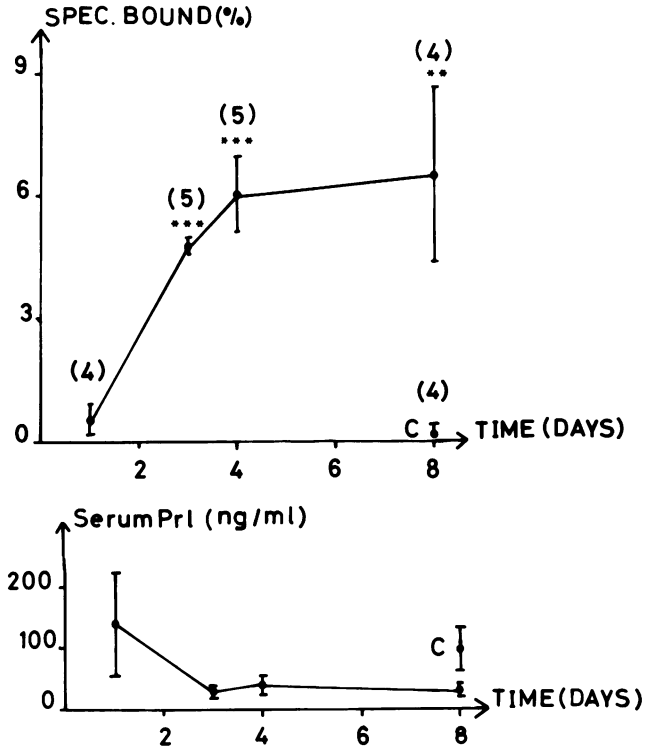

FIGURE 3. Time studies of (*a*) the effect of anterior hypothalamic deafferentation on hepatic hPrl receptors in male rates (specific binding in per cent of the total radioactivity vs. the number of days after deafferentation) and (*b*) serum Prl in animals following deafferentation (Prl concentration is given in ng NIAMDD-RP-1/ml). Control groups of intact males were included at 8 days. The numbers within brackets indicate the number of animals assayed. The double asterisk (**) indicates $p^* < 0.01$; the triple asterisk (***) indicates $p^* < 0.001$ compared to control males.

the female level. The induction of hPrl receptors was evident 3 days following the operation with a maximum after 4 days (Fig. 3). The rise in the concentration of hPrl receptors in the liver was not accompanied by an increase in serum Prl (Fig. 3). Scatchard analysis showed that the induced receptors in the lesioned male rats had similar ligand affinity as the receptors in control female rats (K_D values 0.6 and 0.5 × $10^{-9}M$, respectively). Similar binding capacities were also seen in lesioned males and control females (81 and 102 fmole/mg, respectively).

In order to further localize the "receptor-regulating" center(s) in the brain, transections were performed at a more anterior level. The induction of hepatic hPrl receptors following a lesion at the retrochiasmatic level was not seen in animals with the more anterior transection.

The results presented indicate that hypothalamic

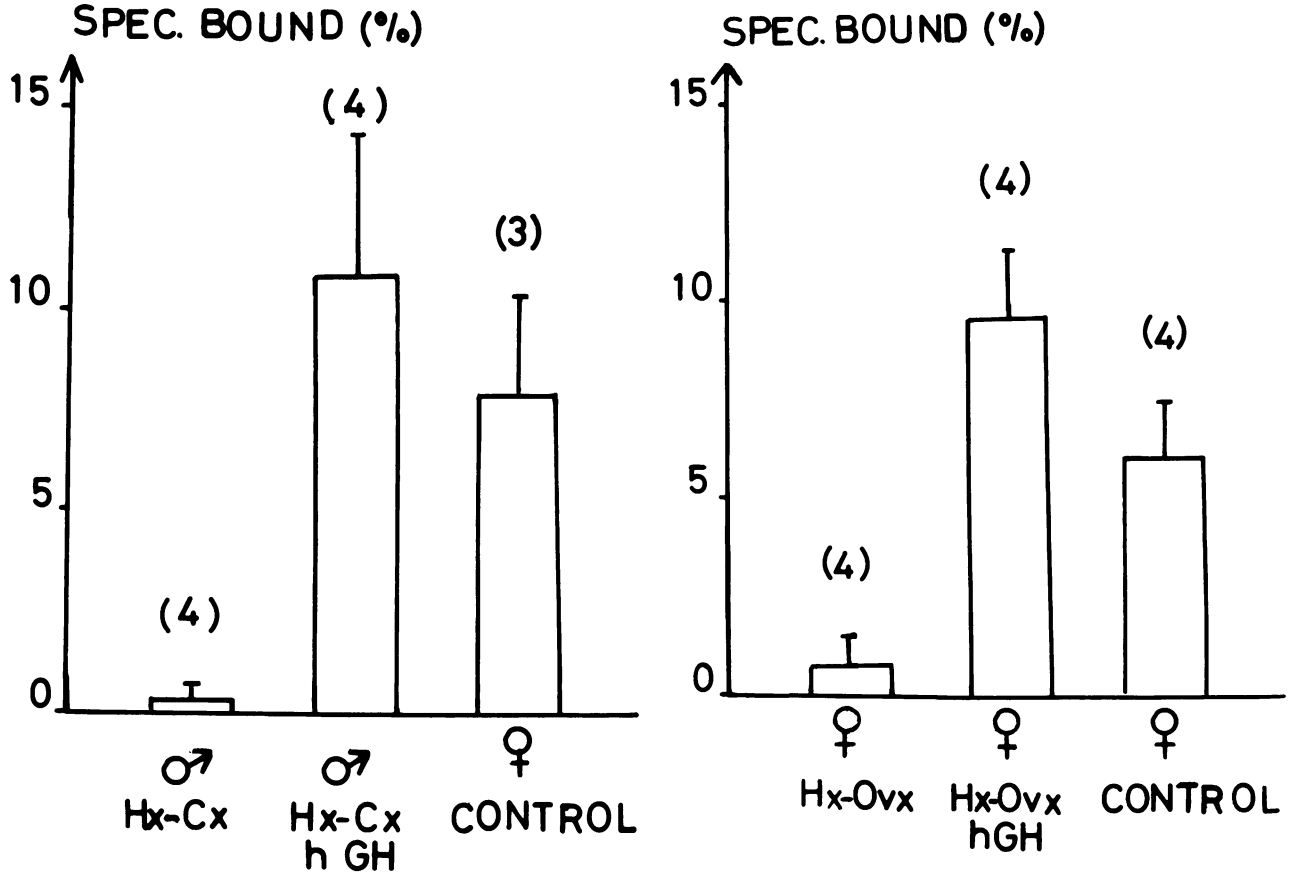

FIGURE 4. Effect on hepatic Prl receptors in hypophysectomized-gonadectomized rats of hGH administered in minipumps at a concentration of 5 μ/μl: (*a*) male hypophysectomized and castrated (Cx) rats; (*b*) female hypophysectomized and ovariectomized (Uvx) rats. Male and female rats were hypophysectomized (Hx); one week later the rats were gonadectomized and, in the cases indicated, given osmotic minipumps with hGH. After one week, the liver Prl receptors were measured. Included is also a control group of intact female rats. On the ordinate is the specific ^{125}I-hPrl binding expressed in per cent of totally added radioactivity. Numbers within brackets indicate number of animals in each group.

deafferentation at the retrochiasmatic level severs the connection between an inhibitory center in the brain which under normal conditions depresses pituitary secretion of a receptor-inducing factor in the male. The exact localization of this brain center remains to be determined. However, since no effect was seen when transections were placed rostral to the suprachiasmatic nucleus, this suggests that the suprachiasmatic and preoptic area may be essential for the control of induction of hPrl receptors. It remains to be elucidated whether a "center" is located in the area between the two levels of transections or whether this area is traversed by passing fiber systems coming in from the lateral aspects to turn caudally towards the basal hypothalamus.

The nature of the hypophyseal receptor-inducing factor is uncertain. Retrochiasmatic deafferentation is likely to interfere with the LH-RH system; destruction of the suprachiasmatic nucleus has been reported to inhibit the phasic component of the LH release (*89*). In view of the finding that castration increases the Prl receptors (*90*), part of the hepatic hPrl receptor induction following deafferentation could possibly be a castration-like effect. However, it is not likely that the large increase of hPrl receptors after deafferentation could solely be a castration-like effect, since castration alone does not increase the Prl receptors to a female level (*91*). The finding that high liver hPrl receptor levels were not accompanied by high serum Prl levels (Fig. 3), does not agree with earlier suggestions that Prl could be the inducing agent of its own receptor.

Using Alzet osmotic minipumps (Alza corporation, Palo Alto, Cal) (*92*) in the study of hepatic Prl

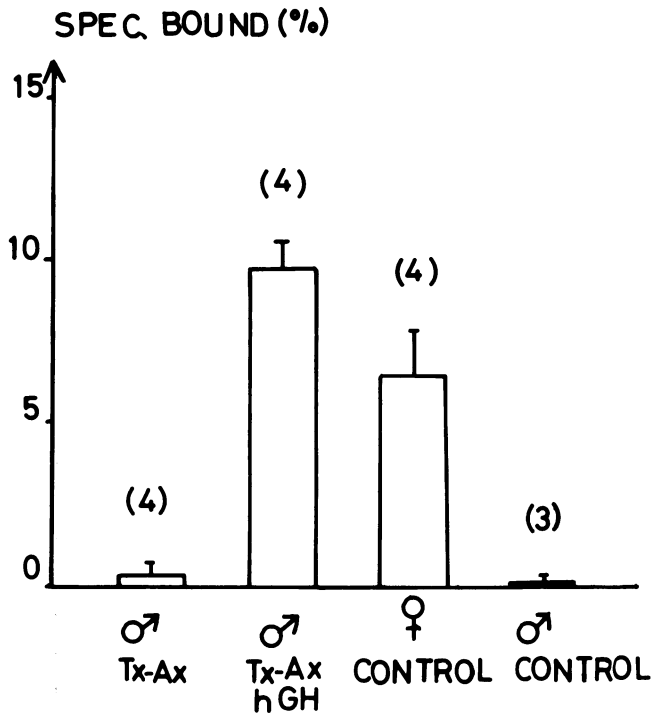

FIGURE 5. Effect of hGH on hepatic Prl receptors in adrenalectomized and thyroidectomized rats. Male rats were thyroidectomized (Tx) and adrenalectomized (Ax); the same day some rats received minipumps containing 5 μg hGH/μl. After one week, liver Prl receptors were measured and serum collected for TSH analysis. Specific ^{125}I-hPrl binding is shown on the ordinate. Numbers within brackets denote number of animals in each group. Serum Tsh was: in ♂ Tx-Ax 1848 ± 654 μg/ml, in ♂ Tx-Ax-hGH 1968 ± 349 μg/ml, in control ♀ 438 ± 99 μg/ml, in control ♂ 606 ± 317 μg/ml.

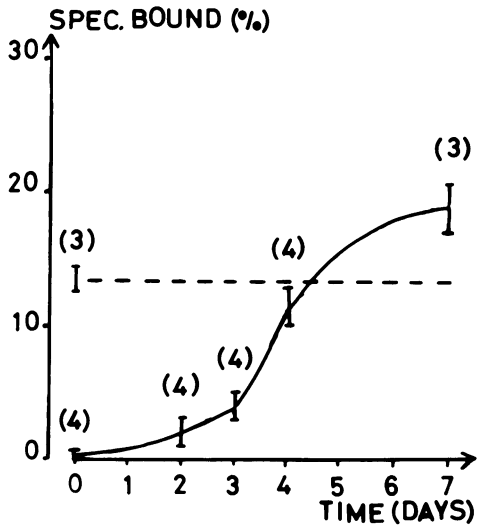

FIGURE 6. Effect of hGH on hepatic Prl receptors as a function of time in male rats. Male animals carried osmotic minipumps containing 5 μg hGH/μl during the time interval indicated. Included is a group of female rats, the Prl receptor level of which is indicated with a dotted line. Numbers within brackets indicate number of animals. Specific ^{125}I-hPrl binding is shown on the ordinate.

receptor regulation we have investigated the effects of hGH, rGH, and rPrl under various experimental conditions. These osmotic minipumps may be placed subcutaneously in the dorsal neck region of the animal and allow a continuous and long-term (7 days) administration of hormone. The pumps have a filling volume of 225 μl and a pumping rate estimated to be 1 μl/hr.

The effect of hGH placed in osmotic minipumps on hepatic Prl receptor levels was tested in hypophysectomized-gonadectomized male and female rats which are known to have low concentration of Prl receptors in the liver (*75, 90*). A group of untreated female rats was included in all of the following experiments. As shown in Figure 4, hGH induced a "feminine" Prl receptor level in livers of hypophysectomized and castrated animals of both sexes.

To ascertain that the effect of hGH was not mediated via the adrenals or thyroids, these organs were removed in male rats. The capacity of hGH to induce a "feminine" hepatic Prl receptor level was unchanged in thyroidectomized-adrenalectomized rats (Fig. 5) when compared to hypophysectomized-gonadectomized animals.

The Prl-receptor-inducing effect of hGH, which apparently did not require the presence of pituitary, thyroid, adrenals or gonads, was then investigated in intact male rats with regard to its time course (Fig. 6). An increase of the receptor concentration to a female level was observed after 4-7 days of hGH administration.

The induced Prl receptors had similar characteristics as the receptors present in control females when calculating data according to Scatchard [K_d = 0.13×10^{-9} vs. $0.15 \times 10^{-9} M$; number of binding sites 88 vs. 57 fmole/mg protein (not shown)].

Figure 7 shows the effect of varying concentrations of hGH in minipumps on receptor levels in male rats. Induction to female Prl receptor levels was seen with 2.5 μg/μl and 5 μg/μl (or 5 mU/μl and 10 mU/μl, respectively).

The effects of rat hormone preparations were then investigated. Rat GH and rat Prl were administered to hypophysectomized male rats in osmotic minipumps (10 μg/μl). A biological effect of this concentration of rGH was indicated by the fact that the animals given rGH increased in weight during 6 days to 114 ± 4% of their initial weight. This may be compared to the weight gain observed

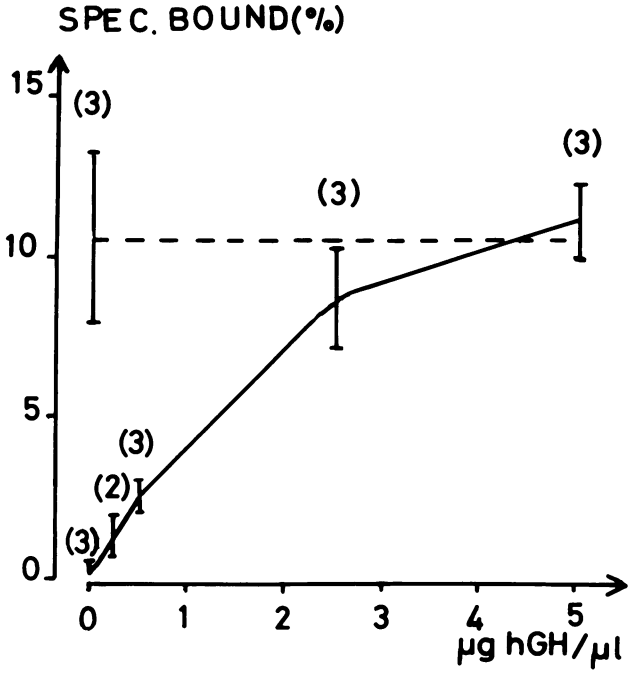

FIGURE 7. Effect of varying concentrations of hGH on hepatic Prl receptors in male rats. hGH, in concentrations indicated on the abscissa, was placed in minipumps which were given to male rats. One week later, Prl receptors were measured. The ordinate shows the specific ^{125}I-Prl binding. Numbers within brackets indicate number of animals in each group. The dotted line shows Prl receptor level in female control group.

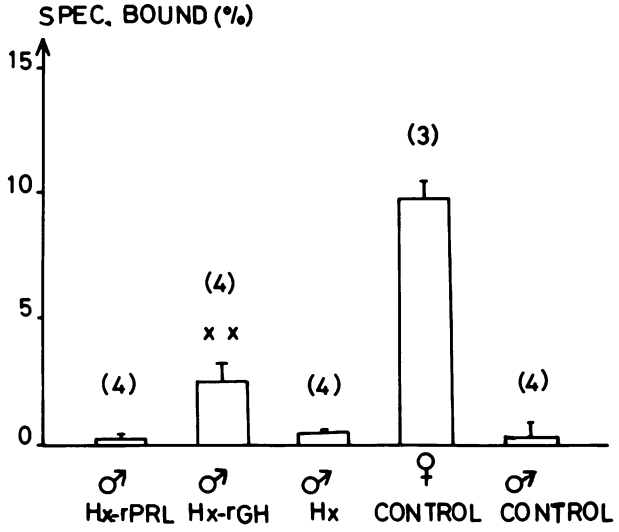

FIGURE 8. Effect of rPrl and rGH on hepatic Prl receptors in hypophysectomized male rats. One week after hypophysectomy (Hx), minipumps with rPrl or rGH, 10 μg/μl, were implanted in the animals of the groups indicated. One week later, Prl receptors were measured. Numbers within brackets indicate number of animals in each group. Groups of intact male and female rats were also included. xx = $p < 0.01$ when compared to Hx male rats.

in hypophysectomized-gonadectomized rats given hGH: 111 ± 4% (from experiment shown in Fig. 4*a*). Hypophysectomized control rats or animals given rPrl showed no weight increase. Administration of rGH to hypophysectomized rats increased serum levels of GH from 22 ± 5 ng/ml to 64 ± 22 ng/ml. Administration of rPrl resulted in serum Prl levels of 28 ± 17 ng/ml as compared to the levels in hypophysectomized controls which were less than 2.5 ng/ml.

Administration of rPrl did not affect hepatic Prl receptor levels in hypophysectomized male rats (Fig. 8). However, some induction of Prl receptors was observed following administration of rGH. Scatchard analysis revealed a specific Prl binding capacity of 33 fmole/mg protein in rats given rGH as compared to 90 fmol/mg in control females; the K_d values were $0.18 \times 10^{-9}M$ vs. $0.15 \times 10^{-9}M$, respectively. Thus, rGH could not induce Prl receptor levels in hypophysectomized rats to the maximal, "feminine" level.

The results presented above indicate that an induction of hepatic Prl receptors can be achieved with hGH administered in osmotic minipumps. The Prl-receptor-inducing activity of hGH was not dependent on the presence of the pituitary, thyroid, adrenals or gonads. The induction time was between 4 and 7 days.

Other workers have failed to see an effect of hGH on hepatic Prl receptor levels, although it was administered three times a day in higher doses than that used in the present investigation (*79*). This may indicate that a continuous hormone administration is necessary for induction. Since, to our knowledge, it has not proved possible to study hepatic Prl receptor induction in an *in vitro* system, osmotic minipumps would seem to be advantageous to use in investigations on induction of Prl receptors. The effect of hGH indicates that either a lactogenic or a somatogenic or a combined principle is responsible for Prl receptor induction. Any definite conclusion as to the nature of the endogenous hormone causing receptor induction cannot be drawn. Due to the small quantities of rPrl and rGH available, we could only test these hormones at one concentration. At this concentration, rPrl was not effective in inducing hepatic Prl receptors. Also previous experiments, where hypothalamic lesions in male rats were found to lead to induction of hepatic Prl receptors without a concomitant increase in serum Prl (*13*, *81*) support the contention that Prl is not the main regulator of Prl receptors in

the liver. The same concentration of rGH as was found to be without effect in case of rPrl, resulted in an increased hepatic Prl receptor level, although not as high as in female rats. This would favor the idea that the inducing hormone is GH or a GH-like peptide. Further studies are required with more purified preparations of rPrl and rGH as well as with combinations of these hormones in order to clarify this matter. Another possibility is that a contaminant of the NIAMDD rGH B_6 preparation is the responsible hormone. In this context, it is interesting to once more draw the attention to the regulation of the sex differences in steroid and drug metabolism in rat liver (*43*). Also here we have proposed a novel pituitary factor acting on the liver (*93*). Considering the many similarities between control of steroid metabolism and Prl receptors, the same pituitary factor might be involved in both cases.

General Comments

The hypothalamo-pituitary-liver axis represents a new concept in endocrine regulation of drug toxicity. The male rat liver has been shown to be more susceptible than the female rat liver to the hepatocarcinogenic action of certain drugs and it is conceivable that sex differences in the metabolic activation of the drugs in the liver may explain the greater sensitivity of male rats to chemically induced hepatocellular carcinoma. Similar sex differences in liver cancer incidence have been reported in the human. Further studies on pituitary-regulated metabolic activation of chemical carcinogens in the liver may lead to a deeper understanding of control of chemical carcinogenesis also in other tissues regulated by pituitary hormones, e.g., the breast and the prostate.

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