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Growth Hormone Regulation of Male-Specific Rat Liver P450s 2A2 and 3A2: Induction by Intermittent Growth Hormone Pulses in Male but not Female Rats Rendered Growth Hormone Deficient by Neonatal Monosodium Glutamate

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SUMMARY

Growth hormone (GH) secretory patterns regulate the expression of several sex-dependent liver cytochrome P450 (CYP) genes. Studies using the hypophysectomized rat model have established that the intermittent plasma GH secretory pattern associated with adult male rats markedly stimulates liver expression of the male-specific CYP 2C11, a testosterone 2α- and 16α-hydroxylase, but is not required for expression of other male-specific liver enzymes, including CYP 2A2, a testosterone 15α-hydroxylase, and CYP 3A2, a testosterone 6β-hydroxylase. In the present study, the effects of intermittent GH treatment on liver CYP expression were studied in adult rats rendered GH deficient by neonatal administration of monosodium glutamate (MSG), which depletes circulating adult GH without the global loss of other pituitary-dependent hormones that is associated with hypophysectomy. Restoration of the normal masculine circulating GH profile of six daily pulses (180–225 ng GH/ml/peak) in MSG-treated male rats by the use of an external pumping apparatus led to a substantial (30–50%) restoration of normal male levels of CYP 2A2 and CYP 3A2 activity, protein, and mRNA. GH pulsation at the nonphysiological frequencies of two or four times per day was less effective unless given at a dose that resulted in supraphysiological plasma GH levels. Although intermittent GH treatment can induce male-specific P450 expression in hypophysectomized female rats, the same hormone treatment did not stimulate CYP 2A2 or CYP 3A2 expression in MSG-treated female rats. Liver GH receptor mRNA levels at adulthood were not significantly altered by neonatal MSG treatment, suggesting that the unresponsiveness of MSG-treated females and the previously reported low responsiveness of MSG-treated males to GH-induced CYP 2C11 expression are not due to the absence of GH receptor. Moreover, normal liver IGF-1 mRNA levels were expressed in the MSG-treated female rats, suggesting that the liver GH receptor is functional in these animals. The present findings establish that the adult male-specific enzymes CYP 2A2 and CYP 3A2 can be positively regulated by intermittent GH pulsation despite their GH-independent expression in hypophysectomized rats. Moreover, neonatal MSG treatment, particularly in female rats, may lead to the loss of factors other than GH that are required for full expression of the pulsatile GH-stimulated CYP 2A2, 3A2, and 2C11 genes.

Steroid hormones and other naturally occurring lipophilic substances serve as important substrates for CYP enzymes found in liver and other tissues (1, 2). Steroid hormones are metabolized by liver P450 enzymes with a higher degree of regioselectivity and stereoselectivity than many foreign compound substrates (3), suggesting that these endogenous lipophiles serve as physiological P450 substrates. The physiological requirements with respect to steroid hormone hydroxylation differ between the sexes, and, not surprisingly, several steroid hydroxylase liver P450s are expressed in a sex-dependent manner (3, 4). Rat P450 enzymes 2C11 and 2C12 are prototypic examples of sex-specific liver P450 enzymes, and they have been a major focus of studies of the underlying endocrine factors, as well as the cellular and molecular regulatory mechanisms that govern sex-specific liver gene expression. CYP 2C11 is the major male-specific androgen 16α- and 2α-hydroxylase in adult rat liver and is induced at puberty in males but not in females (5, 6) under the influence of neonatal androgenic imprinting (programming) (7). In contrast, the steroid sulfate 15β-hydroxylase CYP 2C12 is expressed in a female-specific manner in adult rats (5, 7, 8). Other adult male-specific rat liver P450s include the testosterone 15α-hydroxylase CYP 2A2 and the testosterone 6β-hydroxylase CYP 3A2 (9, 10).

Gonadal steroids do not act directly at the liver to regulate

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ABBREVIATIONS: CYP or P450, cytochrome P450; GH, growth hormone; MSG, monosodium glutamate.

the sex-specific patterns of liver P450 expression. Rather, their effects on liver P450s are primarily mediated via the gonadohypothalamopituitary axis and its sex-dependent regulation of pituitary GH secretory patterns. Plasma GH profiles are sexually differentiated in many species, including human (11, 12), although the differences between the sexes are most dramatic in rodents (13–15). In the adult male rat, GH is secreted by the pituitary gland in an intermittent, or pulsatile, manner that is characterized by high peaks of hormone in plasma (~200–250 ng/ml) every 3.5–4 hr, followed by a period of very low or undetectable circulating GH (<1–2 ng/ml). In contrast, in the adult female rat, GH is secreted more frequently (multiple pituitary secretory events per hour) and in a manner such that the plasma GH pulses overlap and the hormone is continually present in circulation at significant levels (~15–40 ng/ml) at all times (16). Hypophysectomy and GH replacement experiments have demonstrated that these sex-dependent plasma GH profiles are, in turn, responsible for establishing and maintaining the sex-dependent patterns of liver P450 gene expression (4, 9, 17). Thus, in adult female rats, continuous plasma GH stimulates expression of female-specific and female-dominant liver enzymes, such as CYP 2C12, 2A1, and 2C7 and steroid 5α-reductase (8, 18, 19), whereas in adult male rats, intermittent plasma GH pulses induce expression of the male-specific liver enzyme CYP 2C11 and its associated steroid 16α- and 2α-hydroxylase activities (6, 17, 20).

Although it is evident that plasma GH pulses stimulate CYP 2C11 expression in adult rat liver, the effects of these intermittent hormone pulses on other male-specific liver P450s are less clear. Expression of this latter group of P450 enzymes, which includes CYPs 2A2, 3A2, and 2C13, is not obligatorily dependent on plasma GH pulses, as judged from their high level of expression in hypophysectomized rats of either sex (9, 10, 21). On the other hand, expression of CYP 2A2 and CYP 3A2 in adult male rat liver is abolished when neonatal rats are treated with MSG at a dose that is sufficient to damage the arcuate nucleus of the hypothalamus, leading to a permanent loss of circulating GH detectable at adulthood (22, 23). This finding raises the interesting possibility that plasma GH pulses may stimulate expression of CYP 2A2 and CYP 3A2 in adult male rats, even though these P450s are expressed at high levels in hypophysectomized rats. The present study was therefore carried out to elucidate the role of GH in expression of CYPs 2A2 and 3A2 and, in particular, to determine whether restoration of intermittent plasma GH pulses in MSG-treated rats at adulthood restores male-specific CYP gene expression in liver. Our results led us to conclude that despite their high level of GH-independent expression in hypophysectomized rats, the adult male-specific liver CYPs 2A2 and 3A2 can be activated by intermittent GH pulsation given to MSG-treated male rats. These findings, as well as the unresponsiveness of female MSG rats to exogenous GH pulses, are discussed in the context of current models for the regulation of sex-specific liver P450s by pituitary GH secretory profiles.

Materials and Methods

Animals. Male and female Sprague-Dawley rat pups were treated with MSG given as a series of five injections on alternate days for the first 9 days of life, each at a dose of 4 mg MSG/g body weight, according to methods described previously (24) (two to four rats per treatment group, as shown in Table 1). Untreated control rats received an equivalent volume of 1.97 M NaCl diluent (12 μl/g body weight). This dose of MSG results in a loss of detectable circulating GH at adulthood (23, 24), as was verified in the present study (Table 1, *group B*). Rats hypophysectomized at 8 weeks of age were obtained from Charles River Breeding Laboratories. The effectiveness of hypophysectomy was confirmed by the absence of weight gain over a 4–5-week period after surgery and was verified by the absence of detectable circulating GH.

GH replacement in MSG-treated rats and in hypophysectomized rats was carried out with rat GH (National Hormone and Pituitary Program, 1.8 IU/mg; GH dissolved in 30 mM $NaHCO_3$, 0.15 M NaCl containing 0.1 mg/ml rat albumin at a final pH 9.5) by periodic injection via a chronic indwelling right atrial catheter controlled by an external syringe pump (25). Hormone injections were applied as 3-min pulses at frequencies of two, four, six, or seven times per day for 7 consecutive days, at doses of 24–300 ng GH/injection/g body weight, as specified in Table 1. Eight-hour plasma GH profiles based on repetitive blood sampling at 15-min intervals were determined by

TABLE 1

GH peak plasma levels in GH-treated MSG rats

Rats treated with MSG during the neonatal period were given GH pulses (P) by intravenous injection two, four, six, or seven times per day for 7 days (2P, 4P, 6P, and 7P, respectively) or were given GH in two daily subcutaneous injections (2 sc), as described in Methods. The number of individual male and female rats included in each experiment is given in parentheses. Plasma GH peak heights were determined by RIA of sequential blood samples over an 8-hr time period.

Group	Treatment	GH per pulse	Daily GH Dose	Plasma GH	
				Male rats	Female rats
		ng rat GH/pulse/g body weight	*ng rat GH/day/g body weight*	*ng/ml*[a]	
A	Untreated			(4) 217 ± 45[b]	(4) 50 to 90[c]
B	MSG-treated			(2) ≤0–2	(2) ≤0–2
C	+2P	28	56	(3) 254 ± 6	(3) 232 ± 4
D	+4P	28	112	(3) 216 ± 9	(3) 251 ± 27
E	+6P	28	168	(3) 207 ± 18	(2) 234 ± 13
F	+7P	24	168	(2) 200 ± 18	(2) 239 ± 17
G	+4P	49	196	(3) 400 ± 8	(3) 488 ± 11
H	+2P	98	196	(3) 954 ± 11	(3) 700 ± 15
I	+2P	300	600	(3) 1712 ± 14	(3) 1588 ± 13
J	+2sc	98	196	(3) 14 to 32[d]	(3) 12 to 33[d]

[a] Plasma GH peak heights. Values are expressed as mean ± standard deviation for n = three to nine individual peak height measurements (mean ± range for n = two peak height measurements in the case of the two pulses per day treatment groups), except as noted. *b*, *c*, *d*.

[b] Value based on 35 individual plasma GH peak measurements in nine untreated male rats.

[c] Range of plasma GH peak heights observed in 8-hr GH profiles measured in 12 untreated female rats.

[d] Range of plasma GH peak heights during a 3.25-hr period after a single subcutaneous GH injection.

using a radioimmunoassay with a sensitivity of 1–3 ng GH/ml (24). GH peak plasma levels determined by these methods are presented in Table 1. Where indicated, MSG rats were given two daily subcutaneous injections of GH for 7 days at a dose of 98 ng GH/injection/g body weight.

CYP Western blot and enzyme analysis. Microsomes were prepared from individual rat livers and then assayed for individual P450s by Western blot analysis using antibodies for liver microsomal P450s 3A2 and 2A1/2A2 and detection methods described previously (9). P450 form-selective testosterone hydroxylase activities were assayed as described (26). Enzyme activities are reported as mean ± standard deviation (or mean ± range) per milligram of protein for liver microsomes prepared from two to four individual rats per treatment group, as indicated in Table 1. Liver microsomal testosterone 6β-hydroxylase, testosterone 15α-hydroxylase, and testosterone 16α-hydroxylase activities in adult male rats are reflective of the levels of expression of CYPs 3A2, 2A2, and 2C11, respectively (3, 7, 9). The basal levels of each of these testosterone hydroxylase activities found in female rat liver and in MSG-treated male rat liver (see, for example, Fig. 1) are probably due to other CYP enzymes, however, because the corresponding CYP proteins and mRNAs are essentially undetectable in the female and MSG-treated male rat liver when using analytical methods of high sensitivity (e.g., Figs. 2 and 4). The complex nature of the experimental protocol required for these animal studies, involving catheterization, repetitive GH injection, and repetitive blood sampling, precluded our inclusion of the larger number of animals per group, which would be needed for a formal statistical verification of the significance of the dose and frequency dependence of the GH replacement effect in MSG-treated rats. The qualitative differences in the effects of GH on microsomal steroid hydroxylase activities observed in these experiments were readily apparent, however, and are supported by the corresponding Western blot analyses (compare Fig. 1 with Figs. 2 and 3).

RNA analysis. Liver P450 mRNAs were assayed on Northern blots probed sequentially with a series of P450 gene-specific oligonucleotides using hybridization and high stringency washing conditions described previously (27, 28). GH receptor mRNA was detected with the antisense oligonucleotide probe ON-159, 5′-ATC-AGG-GCA-TTC-TTT-CCA-TTC-3′ (hybridization and washing at 45° in buffer containing 0% formamide; see Ref. 27). This probe is complementary to cDNA nucleotides 479–499 of the rat GH receptor cDNA (RATGHR, GenBank Accession No. J04811) (29) and detects as a major signal the mRNA encoding the GH receptor/GH binding protein splice variant and, as a less intense signal, full-length GH receptor mRNA. The relative levels of these two mRNAs were similar in all of the rat liver samples examined in this study. IGF-1 mRNA was detected using a rat IGF-1 cDNA (30) (kindly provided by Dr. Liam Murphy, University of Manitoba). Random prime labeling, hybridization, and high stringency washing were carried out as described previously for S14 cDNA (31). The consistency of RNA loadings between samples was confirmed by ethidium bromide straining of the 18*S* and 28*S* ribosomal RNAs and was verified using an α-tubulin oligonucleotide probe (27).

Results

Male and female rats were treated with MSG during the neonatal period under conditions that led to depletion of circulating GH at adulthood (Table 1, *group B*). MSG-treated male rats expressed low, female-like levels of liver microsomal testosterone 6β-hydroxylase activity (Fig. 1A), a diagnostic marker for the adult male-specific liver P450 enzyme, CYP 3A2 (7). Liver microsomal testosterone 15α-hydroxylase activity, which is catalyzed by another adult male-specific enzyme, CYP 2A2 (9), was also decreased to adult female levels by neonatal MSG treatment (Fig. 1B), as was CYP 2C11-dependent liver microsomal testosterone 16α-hydroxylase activity (Fig. 1C). Western blot analysis verified that the loss of CYP 3A2 and CYP 2A2 protein expression in liver microsomes prepared from neonatal MSG-treated adult rats is complete (Fig. 2B, *lane 3*; Fig. 3B, *lane 3*, see loss of top band, which corresponds to CYP 2A2). In contrast, neonatal MSG treatment had a small stimulatory effect on the female-predominant CYP 2A1 in adult male rats (Fig. 3B, *lane 3* versus *lane 1*, bottom band of doublet); an increase in CYP 2A1-catalyzed liver microsomal testosterone 7α-hydroxylase activity, up to 3-fold, is seen in MSG-treated male rats (22).

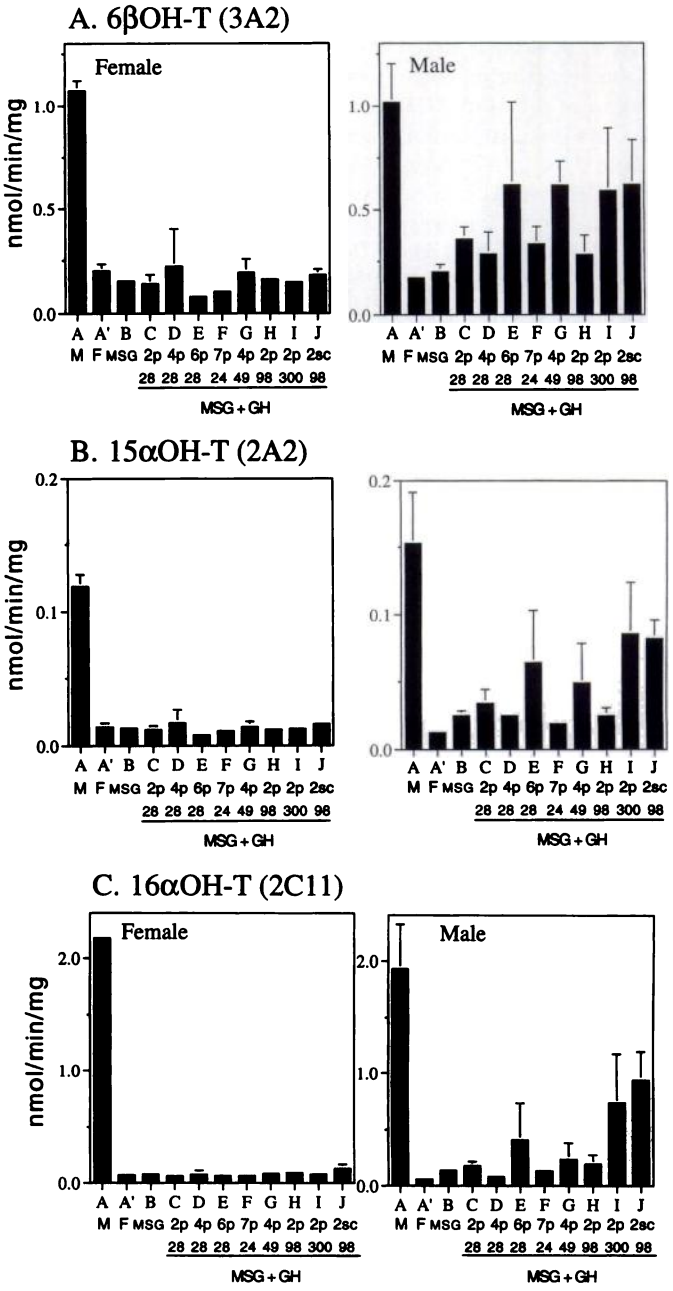

Fig. 1. Influence of neonatal MSG treatment and adult GH replacement on liver microsomal steroid hydroxylase activities. Shown are testosterone hydroxylase activities for liver microsomes isolated from individual female rats (*left* panels) and male rats (*right* panels). Microsomes were assayed for testosterone 6β-hydroxylase activity (CYP 3A2 dependent) (A), testosterone 15α-hydroxylase activity (CYP 2A2 dependent) (B), and testosterone 16α-hydroxylase activity (CYP 2C11 dependent) (C). Treatment groups are those detailed in Table 1.

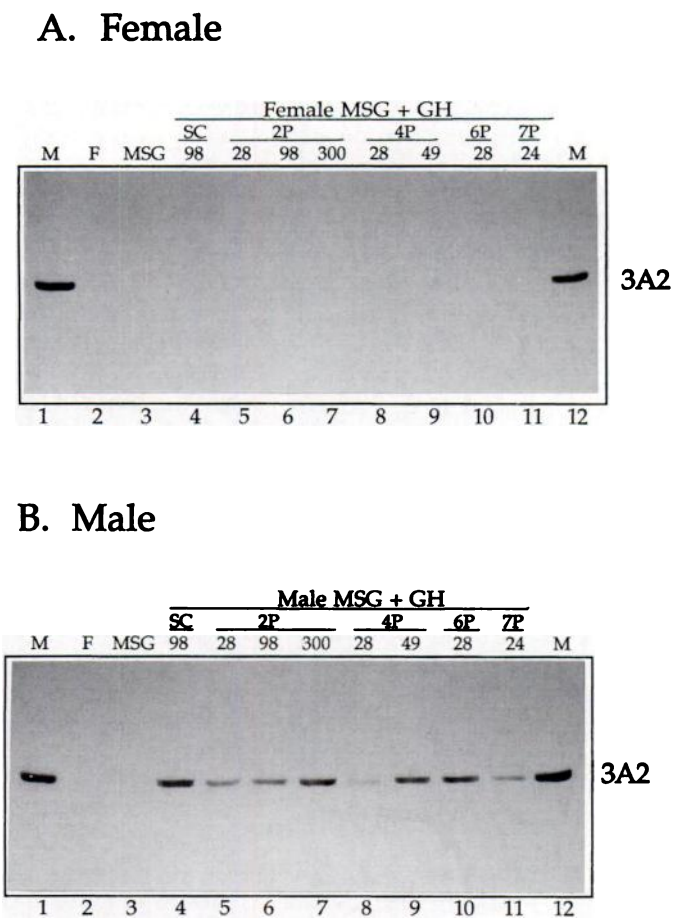

Fig. 2. Liver microsomal CYP 3A2 protein detected in MSG-treated rats. Shown are Western blots of liver microsomes pooled from individual female (A) and male (B) rats constituting each of the treatment groups shown in Table 1. Samples were probed with anti-CYP 3A2 antibody (9).

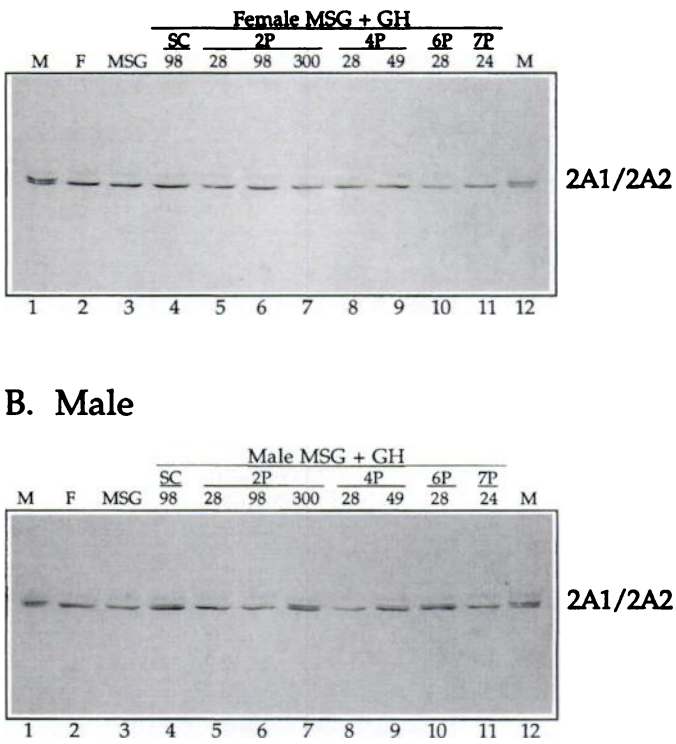

Fig. 3. Liver microsomal CYP 2A1 and CYP 2A2 protein detected in MSG-treated rats. Samples shown in Fig. 2 were electrophoresed on a parallel gel and probed with an anti-CYP 2A1 antibody (9) that is reactive with CYP 2A2 (*top band of doublet in lanes 1 and 12 of A and B*) and with CYP 2A1 (*bottom band of doublet*).

The loss of CYP 2A2 and CYP 3A2 in adult male rat liver after neonatal MSG treatment is consistent with our earlier observations (22) and provided an opportunity to examine directly the effects of plasma GH pulses on adult expression of these two male-specific P450s. GH replacement in MSG-treated male and female rats was carried out by periodic intravenous injections of GH given daily for 7 days with the use of an external pumping apparatus. GH was administered over a range of doses in either two, four, six or seven hormone injections spaced equally over each 24-hr period, as summarized in Table 1. Physiological peak plasma pulse heights of ~200–250 ng GH/ml were achieved when the MSG-treated rats, male or female, were given GH in a dose of 24–28 ng hormone/g body weight (groups C through F). GH injections at ~2-, 3.5–4- and ~10-fold these doses yielded correspondingly higher peak plasma GH levels (groups G through I). In contrast, subcutaneous injection of GH twice daily (a protocol that effectively restores CYP 2C11 expression in hypophysectomized rats; see Ref. 20) resulted in a low level of circulating GH that persisted for several hours (group J).

The effects of each of these GH replacement regimens on liver P450 enzyme and protein levels are shown in Figs. 1–3. Of the GH treatments that yielded physiological peak plasma GH levels (groups C through F, Table 1), GH injection at close to the natural pulse frequency (6 GH pulses/day; group E) was particularly effective at stimulating male-specific liver P450 expression. Thus, significant (albeit incomplete) restoration of liver microsomal testosterone 6β-hydroxylase and CYP 3A2 protein (Figs. 1A and 2B), as well as testosterone 15α-hydroxylase and CYP 2A2 protein (Figs. 1B and 3B), was achieved in male MSG-treated rats given six daily GH pulses. A similar, albeit somewhat less effective, restoration of CYP 2C11-dependent liver microsomal testosterone 16α-hydroxylase activity was achieved with this same hormone replacement protocol (Fig. 1C), a finding that is consistent with our recent observations (32).

Although physiological levels of plasma GH were much less effective or ineffective with respect to restoration of male-specific CYP expression when given at the nonphysiological pulse frequencies of two, four, or seven times per day, GH-stimulated male-specific liver CYP expression was observed when the dose of GH per pulse was increased to nonphysiological levels in the case of the two pulses and four pulses per day protocols (treatment groups G through J). This is seen most clearly in the case of the twice-daily, high-dose intravenous GH protocol and the twice-daily subcutaneous GH replacement protocol (groups I and J).

Adult female rats do not express detectable levels of CYPs 3A2 or 2A2 (or 2C11), as determined using highly specific assays for these adult male-specific liver P450s (Figs. 2A and 3A, *lane 2*) and their mRNAs (Fig. 4A). Although these CYPs could be induced by GH pulses given to MSG-treated male rats, as shown, MSG-treated female rats were unresponsive to intermittent GH pulses, whether given using a physiolog-

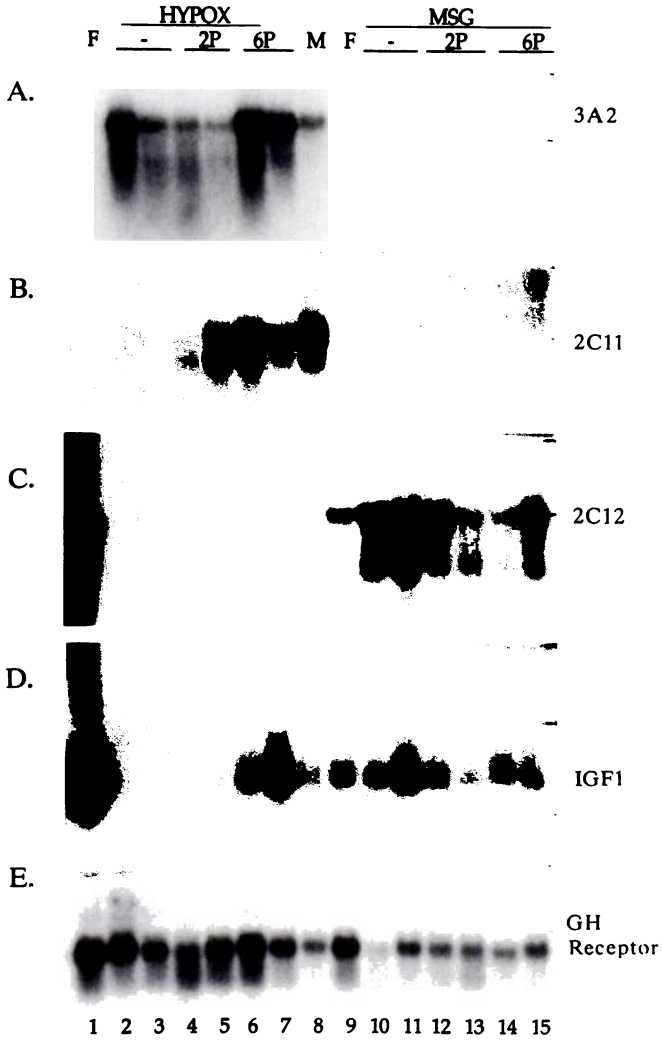

Fig. 4. CYP, insulin-like growth factor-1 (*IGF1*), and GH receptor mRNA expression in hypophysectomized (*HYPOX*, lanes 2–7) and neonatal MSG-treated (lanes 10–15) adult female rats. Shown are Northern blots of individual liver RNA samples (20 μg/lane) probed with gene-specific oligonucleotides complementary to CYPs 3A2, 2C11, and 2C12 and GH receptor mRNA or with IGF1 cDNA, as indicated. RNA samples prepared from untreated adult female rat liver (*F*) are shown in lane 1 (control for samples in lanes 2–7) and lane 9 (control for samples in lanes 10–15). *M*, Untreated adult male liver RNA (lane 8). A, B, and E correspond to sequential probings of a single blot, whereas C and D correspond to sequential probings of the same RNA samples run on a separate blot. The RNA sample analyzed in lane 1 of C and D was loaded at 60 μg instead of 20 μg, and the RNA samples in lanes 7 and 11 of C and D were loaded at 52 and 42 μg, respectively.

ical or a nonphysiological GH replacement protocol. This unresponsiveness of MSG-treated females to induction of CYPs 3A2 and 2A2 (and 2C11) by GH pulses was evident when isolated liver samples were assayed for each P450's enzyme activity (Fig. 1), protein (Figs. 2 and 3), and mRNA (Fig. 4; data not shown). This unresponsiveness of MSG-treated female rats to GH pulses contrasts with the strong responsiveness of hypophysectomized female rats to intermittent GH pulses, which stimulate the expression of CYP 2C11 (Fig. 4B) (20).

To establish whether the unresponsiveness of MSG-treated female rats to exogenous GH pulses is due to an associated loss of GH receptor, we monitored expression of GH receptor mRNA by Northern blot analysis. Fig. 4E shows that liver GH receptor mRNA is expressed in both MSG-treated and hypophysectomized female rats and is not substantially changed in either of these groups after GH replacement. Liver GH receptors appear to be functional in these MSG-treated rats, as judged by the expression of IGF-1 mRNA, which is transcribed in the liver in response to GH stimulation. Interestingly, although liver IGF-1 mRNA is depleted after hypophysectomy and plasma GH replacement induces its restoration (Fig. 4D, *lanes 2 and 3* versus *lanes 4–7*), neonatal MSG treatment did not lead to a loss of liver IGF-1 mRNA in adult female rats (*lanes 10 and 11* versus *lane 9*). Thus, although circulating GH levels in MSG-treated female rats are at most very low (Table 1, *group B*), they appear to be sufficient to support normal levels of IGF-1 expression in the liver. Consistent with this possibility is our observation that neonatal MSG treatment maintains, rather than eliminates, expression of the continuous GH-induced, adult female-specific CYP 2C12 mRNA (Fig. 4C, *lanes 10 and 11*) and its protein (22), whereas GH depletion by hypophysectomy leads to a major decrease in CYP 2C12 expression (Fig. 4C, *lanes 2 and 3*). Accordingly, the apparent unresponsiveness of MSG-treated female rats to exogenous GH pulses may reflect the overriding influence of a very low level of endogenous circulating GH, which most likely is present in plasma in a continuous female-like pattern.

Discussion

Results of the present study establish that plasma GH pulses can positively regulate the expression of CYPs 2A2 and 3A2. This stimulatory effect of GH was not apparent from earlier studies, which were based on the hypophysectomized rat model (9, 10), in which this positive regulatory effect of GH pulsation is masked. In contrast to the decrease in adult CYP 2A2 and 3A2 expression after neonatal MSG treatment, hypophysectomy of male rats does not decrease levels of these two CYPs; rather, it leads to a small increase in enzyme expression. Moreover, in female rats, hypophysectomy stimulates a major derepression that results in high-level expression of these otherwise male-specific liver P450s, despite the absence of GH pulses. CYPs 2A2 and 3A2 may thus be already maximally expressed in hypophysectomized rats, and consequently, a further stimulatory effect of physiological GH pulse replacement cannot be discerned (20). Although it is now clear based on the present study, which was carried out in rats depleted of circulating GH by neonatal MSG treatment, that liver expression of CYPs 2A2 and 3A2 *can* be strongly stimulated by intermittent plasma GH pulses, it is also apparent that expression of these two male-specific liver P450s is not obligatorily dependent on intermittent plasma GH pulsation; this latter point is demonstrated by their high-level expression in hypophysectomized rats of both sexes. This situation contrasts with that of the male-specific CYP 2C11, which is not only positively regulated by plasma GH pulses but is also fully dependent on GH pulses for expression, as is indicated by the very low or undetectable level of CYP 2C11 mRNA in both hypophysectomized and MSG-treated male and female rat liver (Fig. 4B) (20).

The derepression of liver CYP 2A2 and CYP 3A2 expression after hypophysectomy observed in our earlier studies (9) (see Fig. 4A) is likely to result from the loss of multiple

pituitary-dependent factors after ablation of the pituitary gland. In the case of females, these factors include (a) continuous plasma GH, which exerts a significant, albeit only partial, suppression of the high expressed levels of CYPs 2A2 and 3A2 in hypophysectomized rats (33), and (b) thyroxine, which acts in synergy with continuous plasma GH to effect full suppression of both CYP enzymes (28, 33). Thus, the absence of CYPs 2A2 and 3A2 in intact adult female rat liver can be explained by the strong synergistic suppression of these P450s by continuous GH and thyroxine, whereas the high-level expression of these enzymes in intact adult male rats (partially suppressed by normal thyroxine levels) is likely to reflect the strong positive effects that are exerted by intermittent plasma GH pulsation, as revealed by the present study. This stimulatory effect of GH pulses may also help to explain the absence of CYPs 2A2 and 3A2 in MSG-treated male rats at adulthood (see also Ref. 22), since neonatal MSG treatment interferes with the neonatal androgen-dependent hypothalamic imprinting of adult pituitary GH secretory patterns (34, 35) that are presently shown to stimulate expression of these CYPs during normal adult male life without an associated loss of other suppressive pituitary-dependent factors, such as thyroxine (22).

Depletion of assayable plasma GH by neonatal MSG treatment did not prevent the normal expression of CYP 2C12 mRNA in adult female rats (Fig. 4C), which is in agreement with our previous findings at the protein level (22). These results are in contrast to studies in hypophysectomized rats, which reveal a clear dependence of CYP 2C12 expression on continuous GH exposure (8, 19). This apparent inconsistency may be explained in several ways. First, although hypophysectomized and MSG-treated rats are both depleted of circulating GH, the deficiency is limited to adulthood in the former, whereas in the MSG-treated rat, GH is absent throughout the life of the animals, including the critical period of differentiation of the hypothalamic-pituitary-hepatic axis. Because adult expression of each of the sex-dependent P450s, including CYP 2C12, is predetermined by neonatal hormone imprinting (7, 9), it is possible that the absence of GH early during postnatal life disrupts normal liver differentiation, leading to the development of a regulatory mechanism for CYP 2C12 expression that is GH independent. Alternatively, MSG-treated female rats may not be totally devoid of GH but may actually secrete GH at levels that are too low for detection in our radioimmunoassay. Although the hypophysectomized rat has no pituitary, the MSG-treated rat does, albeit one that contains greatly reduced levels of GH (24). However, MSG-treated male rats are unable to secrete pituitary GH due to MSG-induced lesions in the arcuate nucleus, the source of growth hormone-releasing hormone (34, 35). It is possible, therefore, that very low levels of GH may leak from the pituitary in our MSG female rats, giving a low, but continuous plasma GH profile. We have found that plasma from MSG-treated rats consistently displaces a very small amount of radioactive GH ligand from its specific antibody in our radioimmunoassay, suggesting the possible presence of a very low plasma level of GH in these rats. Unfortunately, the displacement is so small that it extrapolates below the sensitivity of the assay (0–3 ng/ml) and cannot be validated statistically. However, if low levels of GH are circulating in these MSG-treated rats and only a small proportion of physiological GH levels are required for CYP 2C12 expression (36), it is possible that they alone, or in combination with other hormones believed to potentiate the inductive effects of GH on CYP 2C12 expression (e.g., insulin, IGF-1, glucagon, and thyroid and/or glucocorticoid hormones) (37), are sufficient to maintain normal CYP 2C12 expression. In this regard, it is far more likely to find normal levels of these potentiating hormones in the MSG-treated rats than in the globally hormone-deficient hypophysectomized rats.

The underlying hormonal basis for the striking difference in the responsiveness of male and female MSG-treated rats to intermittent GH replacement observed in this study is uncertain. The unresponsiveness of the adult female rats is unlikely to reflect sex-dependent differences in the expression of GH receptor in these livers, since GH receptor mRNA is present at similar levels in MSG rats of both sexes and is itself unaffected by GH replacement (Fig. 4E; data not shown). The ineffectiveness of GH pulses with respect to induction of CYPs 2A2 and 3A2 in female, but not male, MSG-treated rats is analogous to the unresponsiveness of MSG-treated female rats to intermittent GH-stimulated CYP 2C11 expression that was seen in our recent studies (32) and confirmed in our present investigation at the level of CYP 2C11 mRNA (Fig. 4B) and CYP 2C11-dependent testosterone 16α-hydroxylase activity (Fig. 1C). Possible explanations for this unresponsiveness of MSG-treated female rats to pulsatile GH-induced expression of the adult male-specific P450s include (a) the absence of factors, other than GH, such as Stat proteins (38), that may be necessary for GH-stimulated liver CYP gene expression and (b) the possibility that MSG-treated female rats contain a very low circulating level of continuous GH that not only supports CYP 2C12 expression, as discussed, but strongly antagonizes any stimulatory effect that exogenous GH pulses may have on male-specific liver CYP gene expression. The K_d of the GH/GH receptor complex, $\sim 10^{-10}$ M, corresponds to only ~2 ng/ml (39), a level that would be difficult to detect in our MSG-treated female rats but might exert a substantial biological effect. If this latter explanation is correct, then presumably MSG-treated male rats, which do respond to exogenous GH pulses, either do not "leak" GH from the pituitary into plasma or, alternatively, release GH at a level or in a temporal pattern that does not antagonize the effects of GH pulsation.

Although the MSG-treated male clearly responds to GH pulses by induction of CYPs 2A2, 3A2, and 2C11, the expression of these enzymes is only partially restored by GH treatment in the MSG-treated male. CYP 2C11 appears to be the least responsive of the three male-specific CYPs, as many of the GH replacement regimens that we used were completely ineffective in stimulating CYP 2C11 expression in MSG-treated males, and a physiological, six-daily GH pulse treatment increased this CYP to only ~25% of normal male levels (Fig. 1C). This observation is consistent with our earlier conclusion, based on the poor response of CYP 2C11 to GH pulses in MSG rats, that the developmental abnormalities produced by neonatal MSG do not result from GH deficiency *per se* but are due to an irreversible loss of sensitivity of the target cell to GH (32). The present study makes it clear, however, that in the male rat, this loss of GH responsiveness is only partial, as evidenced by the substantial, albeit still incomplete, restoration of normal male CYP 2A2 and 3A2 levels after pulsatile GH treatment. Regardless of the magnitude of the induction response, however, our findings es-

tablish that exogenous GH pulses at a physiological pulse height and frequency can stimulate the expression of two distinguishable classes of male-specific liver P450s, i.e., the CYP 2C11 class, which is obligatorily dependent on GH pulses for expression, and the CYP 2A2/CYP 3A2 class, which although stimulated by GH pulses, as demonstrated in the present study, is not obligatorily dependent on GH pulsation for expression. The male-specific CYPs 2C13 and 4A2 may also fall into this latter class of P450s, as is suggested by their up-regulated expression in livers of hypophysectomized rats (21, 40) in a manner that is quite similar to that of CYPs 2A2 and 3A2 (9, 10).

The present demonstration that intermittent plasma GH can stimulate expression of multiple male-specific liver P450 enzymes suggests that despite clear differences in the responses of these CYPs to MSG treatment versus hypophysectomy, common mechanistic features may underlie the GH pulse-driven, male-specific expression of each of their genes. In the case of CYPs 2A2 and 2C11, as well as a third male-specific P450, CYP 2C13, pulsatile GH induces gene expression at the level of transcription initiation (41, 42). Potential DNA regulatory sites that may interact with GH-regulated nuclear factors have been detected in the case of CYP 2C11 (41, 43), but their functional significance is uncertain at this time. Recent studies, however, have identified a liver nuclear transcription factor belonging to the Stat family of proteins (44), designated liver Stat 5, whose tyrosine phosphorylation, nuclear localization, and DNA-binding activity are activated in rat liver in response to intermittent but not continuous plasma GH stimulation (38). Further studies are required to elucidate the role played by this and other factors in the induction of a male liver pattern of P450 gene expression in response to plasma GH pulses.

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