Cooperative Regulation of CYP2C12 Gene Expression by STAT5 and Liver-specific Factors in Female Rats*

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The purpose of this study was to clarify the mechanism(s) responsible for the growth hormone (GH)-induced expression of the CYP2C12 gene. To identify a functional GH-responsive element (GHRE) in vivo, we performed the direct injection of promoter-luciferase chimeric genes into female rat livers. The results showed that the luciferase activity was decreased to approximately 20% by the deletion of the sequence between nucleotides −4213 and −4161. Within this region, two copies of a possible GHRE were present. The sequence of the GHRE was overlapped with that of an interferon-γ-activated sequence, known to be recognized by the signal transducer and activator of transcription (STAT) proteins. In fact, a supershift assay showed that STAT5 was capable of binding to the core sequence of the GHRE. Furthermore, a luciferase assay with reporter plasmids, Δ−4161/−3781, mutated hepatocyte nuclear factor-4 (HNF-4), and mutated HNF-6, revealed that the GH-stimulated expression of the CYP2C12 gene was regulated cooperatively by STAT5, HNF-4, HNF-6, and the factor(s) that binds to the elements, 2C12-I (−4095 to −4074) and 2C12-II (−4072 to −4045). The cooperative regulation by STAT5 and the liver-enriched transcription factors account for the GH-dependent and the liver-specific expression of the CYP2C12 gene in female rats.

CYP2C12 is known to be one of the steroid hydroxylases and is constitutively expressed in female rats but not in male rats (1–4). The sex-specific expression of the CYP2C12 gene is regulated by the plasma growth hormone (GH)1 pattern (5–12). Although the pattern of GH secretion in male rats is characterized by the peaks of large amplitude every 3–4 h with undetectable levels in interpulse periods, the pattern of GH secretion in female rats shows more frequent oscillation of small amplitude (13, 14). Thus, the transcription of the CYP2C12 gene is thought to be activated by the constant level of GH seen in female rats (9, 10, 12).

The effects of GH are mediated by a GH receptor, which belongs to a cytokine/hematopoietin receptor superfamily (15). On the basis of a proposed pathway for the induction of gene expression by GH, the binding of GH to the GH receptor promotes the association of the GH receptor with Janus kinase 2 and the tyrosyl phosphorylation of the Janus kinase 2. (16). Subsequently, the activated Janus kinase 2 phosphorylates the tyrosine residues of the STAT protein. After the formation of the homodimer of the STAT protein or the heterodimer of the STAT protein with other factor(s) in the cytoplasm, the complex translocates to the nucleus and then binds to target sequences (17). Among the STAT family, STAT1, STAT3, and STAT5 have been identified as GH-stimulated proteins (15, 18). Particularly, STAT5 (19) has been reported to participate in the GH-related expression of some genes including c-fos (20), serine protease inhibitor 2.1 (21), and the acid-labile subunit gene (22). Pulsatile plasma GH secretion seen in male rats but not in female rats has been shown to activate STAT5 in the liver (23, 24). Based on these lines of evidence, STAT5 has been regarded as the male-specific regulator for the expression of genes including the sex-limited protein gene (25) and CYP3A10 (26).

To date, the mechanism responsible for the GH-dependent activation of the CYP2C12 gene in females has been studied in vitro. HNF-6 has been reported to be involved in the GH-dependent transcription of the CYP2C12 gene (27). Additionally, it was found that the female-enriched GH-dependent complex, termed GHNF, bound to five distinct regions within the CYP2C12 promoter region between nucleotides −1560 and +60 (28). However, it has not been clarified as yet whether or not the above factors play a key role in the in vivo expression of the CYP2C12 gene. In addition to the uncertainty of the in vivo role of these factors, a functional GH-responsive element(s) has not yet been clarified in the regulatory region of the CYP2C12 gene.

To identify a functional factor(s) responsible for the modulation of the GH-dependent transcription of the CYP2C12 gene, we attempted to search the cultured cells to show the inducibility of CYP2C12 by GH. However, no suitable cultured cells were found. Recently, it has been reported that the direct injection of plasmid DNA into the liver is a useful method to assay the in vivo activity of the CYP2B and CYP2C promoters (29). Thus, we employed the direct injection method to search for a critical factor(s) responsible for the GH-dependent expression and to determine the relative contribution of each transcription factor to the GH-dependent expression of the CYP2C12 gene.

In the present study, we provide evidence that the GH-de-
pendent and liver-specific expression of the CYP2C12 gene in female rats results from a cooperative regulation with STAT5, HNF-4, HNF-6, and factors that bind to elements 2C12-I and 2C12-II in the upstream region of the CYP2C12 gene.

MATERIALS AND METHODS

Animal Treatments—Adult female Harlan Sprague Dawley rats (8 weeks old; Sankyo Experimental Animals, Tokyo, Japan) were used. When necessary, female rats were hypophysectomized at 6 weeks of age. Recombinant human methionylated GH (Somatonorm, Kabi Vitrum, Stockholm, Sweden) was kindly supplied by Sumitomo Pharmaceutical Co. (Osaka, Japan). Human GH was administered by a continuous subcutaneous infusion (0.94 IU/kg/day), which mimics female-type GH secretion, with an osmotic minipump (model 2001, Alza, Palo Alto, CA) or by a pulsatile injection (0.94 IU/kg/day), which mimics male-type GH secretion (10) for 6 days.

Isolation of the 5'-Flanking Region of the CYP2C12 Gene—To isolate the 5'-flanking region of the CYP2C12 gene (30), a gene library prepared from the rat genomic DNA, which had been cleaved by Sau3A I and cloned into AffII vector (Stratagene, La Jolla, CA), was screened with the fragment of a CYP2C12 promoter as a probe. Consequently, a positive clone containing the 9–10-kilobase fragment of the CYP2C12 gene was obtained. The clone was digested with Xho I and HindIII to obtain a 5'-flanking region between nucleotides −5122 and +113 from the transcription start site (30). The resultant fragment was subcloned to the XhoI/HindIII site of pUC19 (pUC5.2).

Construction of 5'-Deletion Mutants for Luciferase Assay—Luc5132 reporter plasmid was constructed as follows. A HindIII site was introduced downstream of the nucleotide +16 of pUC5.2 by site-directed mutagenesis (pUC5.2 (+16)). pUC5.2 (+16) was then cleaved with XhoI and HindIII. The fragment corresponding to nucleotides from −5132 to +16 was ligated into the unique XhoI–HindIII site of basic vector 2 (Toyooki, Tokyo, Japan). Reporter plasmids, Luc4213, Luc4118, and Luc1944, were constructed by the digestion of Luc5132 with restriction enzymes, HindIII, BamHI, and BamHI, respectively. Luc4200, Luc4187, and Luc4161 were generated by polymerase chain reaction using S-4200, S-4197, or S-4161 as a 5'-primer and AS-3614 as a 3'-primer. Synthesized fragments were digested with Bgl II at position −3780 in the CYP2C12 gene. Resultant fragments were inserted into the Smal/Bgl II site of Luc7380. To construct Luc4200ΔGHRE2 without the region between nucleotides −4174 and −4162, the region corresponding to nucleotides from −4200 to −4175 was inserted into the KpnI site of Luc161. To confirm the construction of GHRE to GH-dependent transcription activity, reporter plasmids, 1×GHRE–LucTK, 2×GHRE–LucTK, 4×GHRE–LucTK, and GHRE–Luc1944 were constructed. TK promoter was inserted into the Bgl II/HindIII site of basic vector 2 (LucTK). 1×GHRE, 2×GHRE, or 4×GHRE was then introduced into the XhoI site of LucTK. The copy number and the direction of the oligonucleotides inserted into the reporter plasmids were confirmed by a sequence analysis (31). To construct Luc4200Δ−(4161/−3781) or Luc4187Δ−(4161/−3614), the region corresponding to nucleotides from −4200 to −4162 was inserted into the KpnI site of Luc7380 or Luc4118. To construct Luc4200Δ−(3781/−4144), Luc4200Δ−(1939/−536), Luc4200Δ−(535/−81), Luc4200Δ−(4122/−4036), or Luc4200Δ−(4030/−3781), Luc4200 was digested with Bgl II/EcoR I, EcoRV/BamHI, BamHI/StuI, StuI/Msal I, BamHI/Hpa II, or Hpa II/Bgl II, respectively. Resultant fragments of Luc4200ΔGHRE2 were blunt-ended and self-ligated. To construct GHRE–Luc3780, the region corresponding to nucleotides from −4200 to −4162 was inserted into the KpnI site of Luc7380. To construct GHRE–Luc2C12-I/Luc3780 and GHRE–2C12-II/Luc3780, the regions corresponding to nucleotides from −4905 to −4074 (2C12-I) and from −4072 to −4045 (2C12-II) were cloned into the GHRE–Luc3780. To construct GHRE–2C12-I/Luc3780, the region corresponding to nucleotides from −4905 to −4074 (2C12-I) and from −4072 to −4045 (2C12-II) was cloned into the GHRE–2C12-I/Luc3780.

Preparation of Rat Liver Nuclear Extracts—Nuclear extracts were

FIG. 1. Identification of GH-responsive regions in the CYP2C12 gene. A, effect of hypophysectomy or GH administration on luciferase activity in the liver transfected with Luc5132. A Luc5132 deletion mutant was injected into the livers of nontreated (NT), hypophysectomized (Hypox), and GH-treated (GH) female rats. Luciferase assay with the 5'-deletion mutants of the 5'-flanking region of the CYP2C12 gene. The construction of deletion mutants is described under "Materials and Methods." The numbers given to the deletion mutants indicate the 5’-ends of the 5’-flanking sequence of the CYP2C12 gene counted negatively from a transcriptional start site. These mutants were injected into the liver of adult female rats after GH administration. All values represent the mean ± S.D. from independent experiments shown in parentheses. The data are expressed as the ratio of the luciferase activity of each deletion mutant to the basal activity obtained with Luc5132. *p < 0.05, significantly different between two groups (Mann-Whitney U test).

Role of STAT5a and Liver-specific Factors in CYP2C12 Gene
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RESULTS

Direct Injection of the 5′-Deletion Mutants of the CYP2C12 Gene into Female Rat Livers—To examine whether or not the Luc5132 reporter gene was activated in vivo by GH secreted in a female-type pattern, we injected the DNA of Luc5132 into the liver of female rats (Fig. 1A). As expected, the luciferase activity was detected in the liver transfected with Luc5132. Hypophysectomy of female rats resulted in the abolishment of the luciferase activity. However, the activity was restored to the level seen in intact rats by the continuous infusion of GH to the livers of hypophysectomized female rats. These results indicate that the direct DNA injection is a useful method to clarify the mechanism responsible for the GH-dependent transcriptional regulation of the CYP2C12 gene.

Identification and Characterization of GHREs in the Upstream Region of the CYP2C12 Gene—To identify possible regulatory element(s) involved in the GH-induced expression of the CYP2C12 gene, the 5′-deletion mutants of the gene were constructed as shown in Fig. 1B. When the mutants were injected into livers of female rats, the maximal activity of luciferase was seen in the liver that received Luc4213. The activity was decreased to approximately 20% by the deletion of the nucleotide sequences between −4213 and −4161. Further deletion between −4118 and −1944 also resulted in a significant decrease to a basal activity. These results suggest that at least two regions are necessary for the GH-dependent expression of the CYP2C12 gene in female rats.

The sequence of the 5′-flanking region of the CYP2C12 gene was found to contain 9-bp palindromic sequences (5′-TTCCTAGAA-3′), designated as GHRE1 and GHRE2, in a region between nucleotides −4213 and −4161 (Fig. 2A). Compared with elements reported so far, the sequence of the GHREs overlapped with those of the interferon γ-activated sequence and acute-phase response element (5′-TTCCTAGAA-3′ and 3′-AGCACTTGGCTGAAAGG-5′). The binding of the constitutive factor(s) to the GHREs disappeared with the hypophysectomy of female rats. This result indicates that both GHREs contribute to the GH-dependent activation of the CYP2C12 gene in female rats. Furthermore, a gel shift assay using the GHRE1 or the GHRE2 as a probe showed that a factor(s) in nuclear extracts from female livers bound to each GHRE (Fig. 2C).

Significance of GHREs in the Upstream Region of the CYP2C12 Gene—To determine the significance of GHREs in the GH-dependent expression of the CYP2C12 gene, we compared the effects of GHRE deletion of Luc4213 and Luc4161 on transcriptional activity. The construction of deletion mutants is described under “Materials and Methods.”

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mized female rats. To further confirm the contribution of the GHREs to the GH-dependent expression of the CYP2C12 gene, GHRE-Luc1944, which contains 10 copies of the GHRE, was injected into the liver of female rats (Fig. 3A). The results indicate that the luciferase activity in the liver transfected with GHRE-Luc1944 is much higher than that in the liver transfected with Luc1944. In the livers from hypophysectomized female rats, the activation of the GHRE-Luc1944 was not seen. GHRE-Luc1944 is much higher than that in the liver transfected with Luc1944. The luciferase activity in the liver transfected with GHRE-Luc1944, which contains 10 copies of the GHRE, was significantly increased with the copy number of GHRE (Fig. 3B). Additionally, the luciferase activity with 4×GHRE-LucTK was elevated by the infusion of GH to the livers of hypophysectomized rats (Fig. 3C).

**Binding of STAT5 to the GHRE of the CYP2C12 Gene**—Searching the sequence similar to the GHRE, we found that the sequence of the GHRE overlapped with those of STAT-binding sites (Fig. 4). Particularly, the sequence of the GHRE was completely identical to the STAT5 binding sequence found in the acid-labile subunit gene (22) and interleukin-2 receptor α gene (44). The STAT3 binding sites of the α2-macroglobulin gene (38) and the STAT5 binding sites of the β-casein gene (39) possessed only one base change as compared with the GHRE sequence. To confirm the binding of STAT protein to the GHRE2, we performed a competition assay with STAT-binding sequences seen in the rat β-casein, rat α2-macroglobulin, and human Ly6E and M67 genes (Fig. 5A). We found that the binding of the GH-stimulated factor(s) to the GHRE2 disappeared with the presence of a 20-fold molar excess of the STAT-binding sequences of the β-casein and α2-macroglobulin genes. The competitor of Ly6E and M67 partially inhibited the formation of the complex of the GH-stimulated factor(s) with the GHRE2. Unlike the competitors, the β-casein, α2-macroglobulin, Ly6E, and M67 genes, mutated β-casein, and nonspecific competitor, GATA-1, did not affect the binding of the complex to the GHRE2. To identify a STAT protein to bind to the GHRE, a supershift assay using antibodies to STAT1a, STAT3, STAT5, and STAT5a was performed (Fig. 5, B and C). The results showed that a supershifted band appeared in the presence of antibodies against STAT5 and STAT5a. Thus, it appeared that STAT5a is one of the modulators for the expression of CYP2C12 in the livers of female rats.

**Cooperative Regulation of the CYP2C12 Gene by STAT5 and Other Transcriptional Factors**—The liver-specific expression of CYP2C12 may not be accounted for solely by STAT5, since this protein is also expressed in extrahepatic tissues. Thus, we postulated that liver-specific factors other than STAT5 were required for the expression of CYP2C12 in the livers of female rats. To identify the regulatory regions necessary for the liver-specific expression of the CYP2C12 gene, we performed a luciferase assay with reporter plasmids, Luc4200Δ(−4161/−3781), Luc4200Δ(−3776/−3138), Luc4200Δ(−3137/−1944), Luc4200Δ(−1939/−536), or Luc4200Δ(−535/−81) (Fig. 6A). The deletion of the nucleotides from −4161 to −3781 or from −355 to −81 lowered the transcriptional activity, although the deletion from −3776 to −3138 increased the luciferase activity. The region from −3776 to −3138 may be a negative regulatory region. Within the region between −535 and −81, the putative binding sites of C/EBPα and HNF-4, which is known to be a liver-enriched factor, were present. The mutation within the C/EBPα-binding site did not significantly affect the transcriptional activity (Fig. 6B). However, the mutation of the consensus sequence of HNF-4 decreased the activity to approximately one-twentieth of the level seen with Luc4200. Similarly, it was found that the mutation of the sequence of HNF-6 caused the loss of the transcriptional activity. In addition to
these results, we identified that a novel regulatory region was located in the region between nucleotides −4161 and −3711 (Fig. 6A). Further study using Luc4200Δ (−4161/−4118), Luc4200Δ (−4122/−4036), and Luc4200Δ (−4030/−3781) demonstrated that enhancer elements existed between nucleotides −4122 and −4036 (Fig. 6C). A computer search revealed that this region contained putative CRE (−4095 to −4074) and HNF-3 (−4072 to −4045) sequences. We designated these elements as 2C12-I (−4095 to −4074) and 2C12-II (−4072 to −4045), respectively (Fig. 7A). No factors bound to any region other than 2C12-I and 2C12-II (data not shown). To characterize a factor(s) binding to 2C12-I, a gel shift assay using a mutated probe or competitors was performed (Fig. 7B). The result showed that five factors (I to V) bound to 2C12-I. These factors did not recognize mt2C12-I. Two shifted bands (I and II) disappeared by the addition of a 20-fold molar excess of the consensus CRE sequence. Unlike the CRE, mutated CRE did not affect the binding of the factors to 2C12-II. Furthermore, two shifted bands (II and III) were supershifted by antibodies against CREB1 (Fig. 7B). This result indicates that CREB1 but not CREB2 recognizes 2C12-I. Unknown factors (I, IV, and V) are currently under examination. 2C12-II contained a sequence similar to the binding sequence of HNF-3 (Fig. 7A). To examine whether or not HNF-3 bound to 2C12-II, a gel shift assay with nuclear extracts prepared from the liver of female rats in the presence of antibodies to STAT1α, STAT3, STAT5, and STAT5a was carried out (Fig. 7B). The molecular masses of these binding factors were estimated to be 63, 47.5, 40, 36, or 34.5 kDa. The molecular weights of HNF-3α, -3β, and -3γ have been reported to be 50, 47, and 42 kDa (45), respectively. Although the molecular mass of a factor (47.5 kDa) was similar to that of HNF-3β, other four factors were different from HNF-3. These results indicate that the factors distinct from HNF-3 recognize 2C12-II.

To examine whether 2C12-I and 2C12-II were responsible for the regulation of the CYP2C12 promoter by GH, we constructed deletion mutants with GHRE and 2C12-I or/and 2C12-II (Fig. 7E). Insertion of both 2C12-I and 2C12-II restored the transcripational activity to the level seen in the liver transfected with Luc4200, although the injection of a reporter plasmid carrying 2C12-I or 2C12-II alone did not induce the activity. Taking these results together, we conclude that the GH-dependent expression of the CYP2C12 gene is regulated cooperatively by STAT5, HNF-4, HNF-6, and an unknown factor(s), which binds to 2C12-I and 2C12-II.

Activation of CYP2C12 Promoter by GH Secreted in Male Rats—To examine whether or not the luciferase activity derived from the Luc5132 reporter plasmid was activated by GH secreted in male rats, we injected Luc5132 into the livers of male rats (Fig. 8). Interestingly, it was found that the luciferase activity was detected not only in female rats but also in male rats. The hypophysectomy of rats abolished the luciferase activity. As expected, the pulsatile as well as constant infusion of GH to hypophysectomized rats increased the activity. These results indicate that the pulsatile GH secretion also induces transcription factors responsible for the induction of CYP2C12.

DISCUSSION

In an attempt to study the mechanism responsible for the transcriptional regulation of a certain gene, cultured cells in which the gene expression is seen are generally used, since the cultured cells possess a transcription factor(s) necessary for the gene expression. However, to our knowledge, suitable cultured
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cells that show the inducibility of CYP2C12 by GH have not been found. In addition, it is hard to reproduce the female- or male-type GH stimulation with cells in culture. Therefore, we employed the direct DNA injection system developed by Kemper et al. (29). As expected, a luciferase activity in the liver transfected with Luc5132 was clearly detected. Thus, the direct DNA injection was evaluated to be a useful tool to explore the mechanism of GH-dependent transcriptional regulation.

The binding sites of HNF-4 (33), HNF-6 (27), C/EBPα (32), insulin response element-A-binding protein (46), and GHNF (28) have been reported as the transcriptional regulator of the insulin response element-A-binding protein (46), and GHNF mechanism of GH-dependent transcriptional regulation. DNA injection was evaluated to be a useful tool to explore the DNA injection was evaluated to be a useful tool to explore the mechanism of GH-dependent transcriptional regulation.

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GHREs seem to function synergistically. Supporting this phenomenon, it has been reported that two copies of the STAT5 binding site are required for the maximal induction of serine protease inhibitor 2.1 by GH (21).

The sequence of GHRE, TTCCTAGAA, was identical to that of interferon γ-activated sequence seen in the bovine β-casein (47) and mouse acid-labile subunit genes (22), known to be recognized by both STAT5a and STAT5b. However, STAT5b was reported to be suppressed by GH secreted in female rats but not male rats. In fact, the amount of STAT5b in the female rat livers was estimated to be low (24, 49, 50). Our result of a supershift assay demonstrated that STAT5a is a major regulator of the CYP2C12 gene in females. It has been reported that STAT5a-STAT5b heterodimer was necessary for the expression of female-specific CYP2B enzyme (51). Thus, the heterodimerization of STAT5 proteins may be important for the expression of CYP2C12 in rats and mice.

CYP2C12 is unique in that its expression is female-specific. On the other hand, STAT5 is considered to be a male-enriched transcriptional factor, although we have shown that STAT5 serves as the modulator of CYP2C12 gene expression in female as well as male rats. Actually, the activation of the Luc5132 reporter gene was also seen in male rats (Fig. 8). An unknown suppressor protein(s) involved in the gender-related difference in the expression of the CYP2C12 may be present in male rat livers. Additionally, it is also possible to assume that the CYP2C12 gene is inactivated at a chromatin level in male rats. Since a naked plasmid was transfected into the livers, sex difference in chromatin structure cannot be seen in the present experiments. These possibilities remain to be examined.

GHNF has also been reported to show a sex difference in the expression level (27). However, GHNF-6 functioned as the activator of the Luc5132 reporter gene in male rats (Fig. 8). HNF-6 may not be a critical factor determining the sex-related difference. We demonstrated that HNF-4 was a major factor to regulate the expression of the CYP2C12 gene. Our result does not agree with a previous work (33). Although the transactivation of the CYP2C12 gene by HNF-4 alone was weak (33), HNF-4 was needed for the expression of CYP2C12 when it cooperated with STAT5 and the factors binding to 2C12-I and 2C12-II. In addition, the contribution of C/EBPα to the CYP2C12 expression was small as compared with the results of the previous work (32). This disagreement may be explained as follows. The methodology of direct DNA injection was employed in the present

![Image of regulatory regions coupled with the GHREs](https://example.com/image.jpg)
While primary hepatocytes and cultured cells such as HepG2 were used in the previous work, the C/EBPα expression plasmid was transfected into the cultured cells. Cultured cells transfected with C/EBPα may not reflect the in vivo expression of the CYP2C12 gene. To date, it has been reported that a SRY-like protein inhibits the binding of C/EBPα in cultured cells (46). Since the in vivo function of a SRY-like protein is not analyzed in the present study, we cannot exclude the possibility that a SRY-like protein is related to the in vivo expression of CYP2C12. The regulation of the CYP2C12 gene by a SRY-like protein is currently under examination.

We identified novel regulatory regions, 2C12-I and 2C12-II. CREB is known to be a transcription factor bound to CRE (52) and known to interact with p300/CBP (53), which functions as histone acetyltransferase. In addition to CREB, STAT5 also interacts directly with p300/CBP (54). Therefore, STAT5 may function synergistically with CREB through p300/CBP. It has been reported that Ca2+1 channel blocker inhibits the expression of the CYP2C12 gene in rat hepatocytes (55). The activation of CREB occurs in response to elevated intracellular Ca2+1 through Ca2+/calmodulin-dependent protein kinase (56). Thus, the Ca2+1-dependent expression of the CYP2C12 gene can be explained by the activation of CREB.

In addition to 2C12-I and 2C12-II, negative regulatory region (−3776 to −3138) was present in the upstream region of the CYP2C12 gene. However, we could not find a typical negative regulatory region reported so far. Further study is needed for the identification of a factor(s) involved in the negative regulation.

It has been proven that STAT5 interacts directly with a glucocorticoid receptor (48) or a p300/CBP (54). These components of 2C12-I or/and 2C12-II in GH-dependent activation. The construction of deletion mutants is described under "Materials and Methods." These mutants were injected into the liver of adult female rats. All values represent the mean ± S.D. from independent experiments shown in parentheses. The data are expressed as the ratio of the luciferase activity of each deletion mutant to the basal activity obtained with Luc5132 in nontreated female rats. M, male rats; F, female rats.

![Figure 7](image-url)  
**Fig. 7.** Characterization of 2C12-I and 2C12-II found in the 5′-flanking region of the CYP2C12 gene. A, nucleotide sequences of 2C12-I and 2C12-II. CRE and HNF-3 binding elements are in boldface type. The sites of mutated (mt) sequences are underlined. B, gel shift assay using 2C12-I and mt2C12-I. C, gel shift assay with 2C12-II or mutant probes. To examine the effects of competitors on the binding of HNF-3 to a TTR probe, a competitor DNA, TTR, 2C12-II, or TAT was added at a 25-, 100-, or 250-fold molar excess of the probe TTR. D, Southwestern blot analysis. Nuclear extracts (60 μg) from the liver of female rats were incubated with 32P-labeled 2C12-II or mt2. E, function of 2C12-I or/and 2C12-II in GH-dependent activation. The construction of deletion mutants is described under "Materials and Methods." These mutants were injected into the liver of adult female rats. All values represent the mean ± S.D. from independent experiments shown in parentheses. The data are expressed as the ratio of the luciferase activity of each deletion mutant to the basal activity obtained with GHRE-2C12-I/II-Luc3780. **, significantly different from the transcriptional activity in the liver transfected with GHRE-2C12-I/II-Luc3780 at p < 0.01.
plexes regulate cooperatively the prolactin-dependent expression of the β-casein gene in the mammary gland. We demonstrated in this study that CREB and liver-specific factors together with STAT5 were required for the transcriptional activation of the CYP2C12 promoter by GH. The GH-stimulated and the liver-specific expressions of CYP2C12 in female rats were confirmed as expliable by the cooperative regulation by STAT5, the liver-specific factors, and other factors such as a CREB.

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