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Insulin Is the Essential Factor Maintaining the Constitutive Expression of Hepatic Sterol 14-Demethylase P450 (CYP51)[1]

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The role of serum insulin in regulating the expression level of hepatic sterol 14-demethylase P450 (CYP51) was examined. Administration of streptozotocin, which destroys pancreatic β-cells, caused reduction of CYP51 mRNA level in rats in parallel with the loss of serum insulin. Streptozotocin treatment also reduced the CYP51 activity. The decreased mRNA level and activity of the streptozotocin-treated rats were restored to the normal level within 24 h by repeated administration of insulin. CYP51 level of normal rats was insensitive to the circadian variation of serum insulin and insulin administration, and no significant difference was observed between the hepatic CYP51 activities of Sprague-Dawley and Wistar lean rats, although the serum insulin concentration of the latter was higher than the former. These facts indicate that the expression of hepatic *CYP51* is maintained by serum insulin, and its lowest physiological level is sufficient for supporting the expression of *CYP51*. The responses of *CYP51* expression to streptozotocin and insulin treatments were closely similar to those of the sterol regulatory element binding protein (SREBP)-1c expression [Shimomura *et al.* (1999) *Proc. Nat. Acad. Sci. USA* 96, 13656–13661]. Based on this fact, the possible contribution of SREBP-1c to the insulin-dependent expression of hepatic *CYP51* gene was also discussed.

Key words: CYP51, regulation, insulin, sterol 14-demethylase P450, sterol regulatory element binding protein (SREBP), streptozotocin.

Sterol 14-demethylase P450 (CYP51) is an essential enzyme for cholesterol synthesis in mammals (*1*). The *CYP51* gene has been conserved throughout the evolution of eukaryotes (*2–4*). In mammals, *CYP51* is ubiquitously expressed in every organ (*5*, *6*) and its structure shows the characteristics of a housekeeping gene (*5*, *6*). There is no doubt that the dominant function of CYP51 is the 14-demethylation of a sterol precursor indispensable for sterol synthesis in eukaryotes (*1*), and the above-mentioned unique characteristics of this P450, *i.e.*, high evolutionary conservation and ubiquitous expression, must be due to this essential function. However, it has been pointed out that CYP51 of extrahepatic organs, such as gonads, may contribute to some other functions. Byskov *et al.* (*7*) revealed that the 14-demethylated products of lanosterol acted as meiosis-activating sterols for the cultured mouse follicles, and Yoshida *et al.* (*8*) reported the inductive effect of gonadotropin on rat ovarian CYP51. Rozman *et al.* (*9*) found the essential role of CREMτ, a cAMP-responsive element modulator, in the regulation of *CYP51* expression in male germ cells of rats. These findings suggest that CYP51 of mammals may contribute to multiple functions and its expression may be regulated in an organ-specific manner.

Liver is the dominant organ of cholesterol synthesis, and hepatic CYP51 must dedicate this function. Therefore, the level of hepatic CYP51 may contribute to cholesterol homeostasis of mammals. Liver contains a long sterol-metabolizing pathway spanning from lanosterol to bile acids (Fig. 1). CYP51 and three additional P450s participate in this metabolism, and three of them, CYP51, CYP7A (cholesterol 7α-hydroxylase P450), and CYP8B (sterol 12α-hydroxylase P450), are evolutionarily related species that might be derived from a common ancestral P450, suggesting evolutionary continuity of this pathway (*10*). However, this pathway is functionally divided into two parts, cholesterol synthesis and its catabolism, and these three P450s play key roles in each part (Fig. 1). CYP51 initiates this metabolism by mediating the 14-demethylation of lanosterol. CYP7A catalyzes 7α-hydroxylation of cholesterol, which is the first step of the catabolic part and the rate-limiting step of cholesterol catabolism to bile acids, and CYP8B is the key enzyme causing divergence of the pathway to cholic acid formation. Accordingly, regulation of expression levels of these P450s is considered to be important

[1] Molecular species and genes of P450 are expressed with the "CYP" code given by the recommended nomenclature of P450 superfamily [Nelson *et al.*, *Pharmacogenetics* 6, 1–42 (1996); http://drnelson.utmem.edu/CytochromeP450.html]. CYP51, sterol 14-demethylase P450; CYP7A, cholesterol 7α-hydroxylase P450; CYP8B, sterol 12α-hydroxylase P450; CYP27, sterol 27-hydroxylase P450.

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Abbreviations. CREM, cAMP responsive element modulator; INS, insulin; IRS, insulin response sequence; SD, Sprague-Dawley rat; SRE, sterol regulatory element; SREBP, sterol regulatory element binding protein, STZ, streptozotocin; WF, Wistar fatty rat; WL, Wistar lean rat.

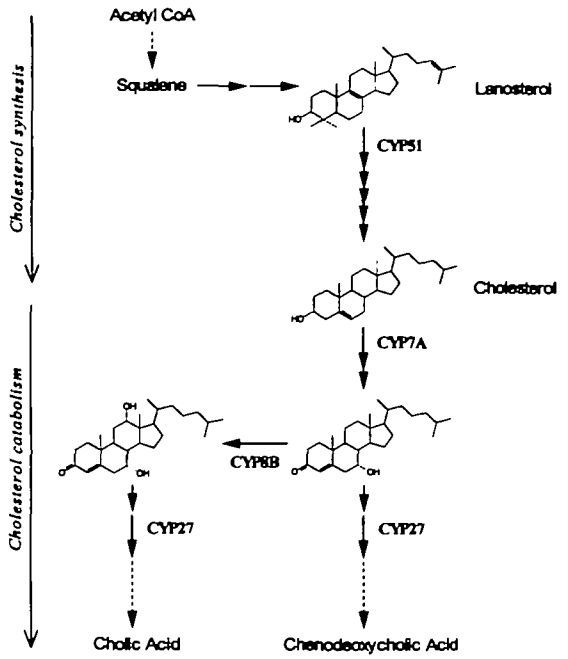

Fig. 1. **Sterol-metabolizing pathway and P450-mediated reactions.** Sterol-metabolizing pathway spanning from lanosterol to bile acids is functionally divided into two parts, synthetic and catabolic pathways of cholesterol. CYP51 mediates the initial reaction of the synthetic pathway, 14-demethylation of lanosterol. CYP7A catalyzed 7α-hydroxylation of cholesterol, which is the initial and rate-limiting step of the catabolic pathway. CYP8B branches the catabolic pathway to cholic acid formation by catalyzing the 12α-hydroxylation of the 7α-hydoxy intermediate. CYP27 is a sterol 27-hydroxylase oxidizing the terminal carbon (C_{27}) of sterol side chain.

for maintaining hepatic cholesterol level. Recently, Ishida *et al.* (*11*) reported that the dominant regulatory mechanism of *CYP8B* is the repression by insulin (INS). They also found that the CYP7A level in H4TG cultured rat hepatoma cells was also repressed by INS (*11*). These findings indicate a suppressive role of INS on cholesterol catabolism. On the other hand, INS is known as a major factor for maintaining hepatic lipid synthesis (*12*) and is reported to elevate the expression level of HMG-CoA reductase (*13, 14*), the rate-limiting enzyme of cholesterol biosynthesis. Accordingly, INS is considered to elevate hepatic cholesterol level by enhancing its synthesis and repressing its catabolism to bile acids. Then, the question arises whether INS affects the level of CYP51, which is the initiating enzyme of the cholesterol synthetic pathway from lanosterol (Fig. 1). CYP51 is an enzyme included in the metabolic pathway proper to sterol synthesis branching from the trunk of the mevalonic acid pathway, whereas HMG-CoA reductase is the key enzyme situated at the root of the trunk. Therefore, CYP51 is a suitable enzyme for regulating cholesterol synthesis independently of other isoprenoid syntheses.

To answer the above questions and to elucidate the regulatory mechanism of hepatic *CYP51* expression, we examined the *in vivo* effects of serum INS concentration to the hepatic CYP51 level in rats. Results indicated that the normal concentration of serum INS is essential for maintaining steady-state level of hepatic CYP51. In addition, it was also found that INS did not affect the expression of testicular *CYP51*.

MATERIALS AND METHODS

Experimental Animals and Treatments—Male Sprague-Dawley (SD) rats (2 months old, 270–280 g of body weight) were maintained on a 12 h light and 12 h dark cycle and fed *ad libitum* normal laboratory rat chow and tap water. STZ (Sigma) dissolved in 0.1 M citrate buffer, pH 4.6, was injected subcutaneously at a dose of 65 mg/kg of body weight. The rats were sacrificed at 18 h, 24 h, 48 h, and 6 d after the STZ administration, and serum INS concentration, hepatic CYP51 mRNA level and CYP51 activity were determined. Bovine INS, which was dissolved in 1.5% glycerol (0.3 mg INS/ml) with minimum amount of HCl, was injected subcutaneously once or five times at 3-h intervals to the STZ-diabetic rats (6th day of the above STZ treatment) at a dose of 8 IU/kg of body weight. Rats were sacrificed 12 h after the last injection, and the above-mentioned parameters were determined. The livers of Wistar fatty (WF) and Wistar lean (WL) rats, which were quickly frozen after isolation, were kindly provided by Dr. Odaka of Takeda Chemical Industries, Osaka.

Preparation of Enzyme Source and Poly(A)$^+$ RNA—The liver isolated from individual rats was cut into small pieces and divided into two portions for preparing microsomes and RNA. The portion used for preparing microsomes was homogenized with 0.1 M potassium phosphate buffer, pH 7.5. The homogenate was centrifuged at 9,000 ×*g* for 20 min, and the supernatant was further centrifuged at 100,000 ×*g* for 60 min. The membrane fraction recovered as precipitate of the latter centrifugation was suspended in 0.1 M potassium phosphate buffer, pH 7.5, and used as the microsomes for determining CYP51 activity. The supernatant of the latter centrifugation was used as S-100 fraction for the microsomal CYP51 assay (see below). RNA was extracted by the guanidine HCl method (*15*) from the remaining portion of the liver. The RNA extracts from the rats belonging to one experimental group were combined and subjected to oligo(dT)-cellulose column chromatography to enrich poly(A)$^+$ RNA (*16*). The testes isolated from a rat were minced after removal of capsules and combined. One portion of the minced testis was homogenized with 0.1 M potassium phosphate buffer, pH 7.5, and centrifuged at 10,000 ×*g* for 10 min. The resulting supernatant was used as S-10 fraction, the enzyme preparation used for the assay of testicular CYP51 activity. Testicular poly(A)$^+$ RNA was prepared as above from the remaining portion of the testis specimen.

Assay of CYP51 Activity of Rat Liver and Testis—CYP51 activity was assayed principally by the same method as described previously (*17, 18*). The reaction mixture for determining hepatic CYP51 activity consisted of lanosterol (47 nmol, dispersed with Tween 80), microsomes (5.0 mg protein), S-100 fraction (10 mg protein), KCN (1 mM), an NADPH-generating system, and 0.1 M potassium phosphate buffer, pH 7.5; and the final volume of the mixture was made up to 2.0 ml. The reaction mixture for determining testicular CYP51 activity was same as above except that the S-10 fraction (8.0 mg protein) was used as enzyme source instead of microsomes and S-100 fraction. The reaction was run aerobically at 37°C for 10 min (liver) or 30 min (testis) under constant shaking. The reaction was terminated by saponification (*17*), and sterols were extracted

with diethyl ether/petroleum ether (5/95). The extracted sterols were separated by TLC (*17*), and the fraction containing lanosterol and its 14-demethylated metabolites was extracted. The extracted sterols were trimethylsilylated and analyzed by GLC (*17–19*). The demethylase activity was calculated from the chromatographically determined conversion ratio (*17–19*) of lanosterol to the two demethylated metabolites (*19*) and the initial amount of lanosterol after correction of the endogenous metabolites by the gas-chromatogram of sterols extracted from the reaction mixture at the time zero of the incubation.

Northern Blot Analysis—Rat cDNA clones used as hybridization probes for CYP51 (*2*, *3*) and glyceraldehyde 3-phosphate dehydrogenase (*10*) mRNAs were prepared as described previously. Poly(A)$^+$ RNA preparations (1–2 μg/lane) were electrophoresed on 1% agarose gels containing 2.2 M formaldehyde as described by Thomas (*20*) and transferred to Nytran membranes (Schleicher & Schuell GmbH, Dassel). After the transfer, the membranes were stained with methylene blue to confirm the equal loading of poly(A)$^+$ RNA on each lane (*21*). The membranes were hybridized with the ^{32}P-labeled cDNA probes in the hybridization solution containing 6× SSC, 5× Denhardt's, 10 mM EDTA, 1% SDS, and 0.5 mg/ml sonicated salmon sperm DNA at 68°C for overnight. The membranes were washed with 0.1× SSC containing 0.5% SDS at 50°C and exposed to Kodak BMX films at −80°C. The radioactivities of the hybridized areas were quantified using Bio-imaging Analyzer System BAS2000 (Fuji Photo Film, Tokyo).

Other Analytical Methods—INS and glucose concentrations in the serum were determined by using Glazyme insulin-EIA test kit (Wako Pure Chemicals, Osaka) and Glucose CII-test kit (Wako Pure Chemicals, Osaka), respectively. Protein was determined by the method of Lowry *et al.* (*22*) with bovine serum albumin as the standard.

RESULTS

Effects of STZ-Treatment on Hepatic CYP51 Level—To estimate the requirement of INS for the expression of hepatic *CYP51* gene, we studied the effects of INS deficiency caused by the STZ-induced necrosis of pancreatic β-cells on the activity and mRNA level of CYP51 in SD rat liver. STZ was injected subcutaneously once into rats (65 mg/kg of body weight), which were sacrificed 18 h, 1 d, 2 d, and 6 d later. Serum glucose concentration was raised to more than 200 mg/dl at 18 h after the STZ injection, and this high serum glucose level was maintained for 6 d. In addition, serum of many but not all STZ-treated rats showed marked turbidity due to lipid droplets after 2 d. These observations indicated that STZ effectively destroyed pancreatic β-cells, and rats showed diabetic symptoms.

Figure 2 summarizes the serum insulin concentration, hepatic CYP51 mRNA level and hepatic CYP51 activity in normal and STZ-treated SD rats. Serum INS concentration was reduced to about 50% of the normal value 18 h after the STZ administration and was decreased to less than 10% of the normal value 2 d after STZ treatment (Fig. 2A). Serum INS continued to show this minimal level 6 d after treatment. This observation clearly indicated that the single administration of STZ effectively and irreversibly destroyed the pancreatic β-cells and diminished the serum INS. The hepatic CYP51 mRNA level was reduced nearly in parallel with the serum INS (Fig. 2B). In this experiment, the application of equal amounts of RNA to each lane was confirmed by the staining with methylene blue (*21*), and no detectable change was observed on the level of glyceraldehyde 3-phosphate dehydrogenase mRNA measured as a reference (data not shown). The change of CYP51 mRNA level observed in the STZ treatment strongly suggested the correlation of hepatic CYP51 mRNA level to the serum INS concentration. These results suggested that serum INS concentration affected the hepatic *CYP51* ex-

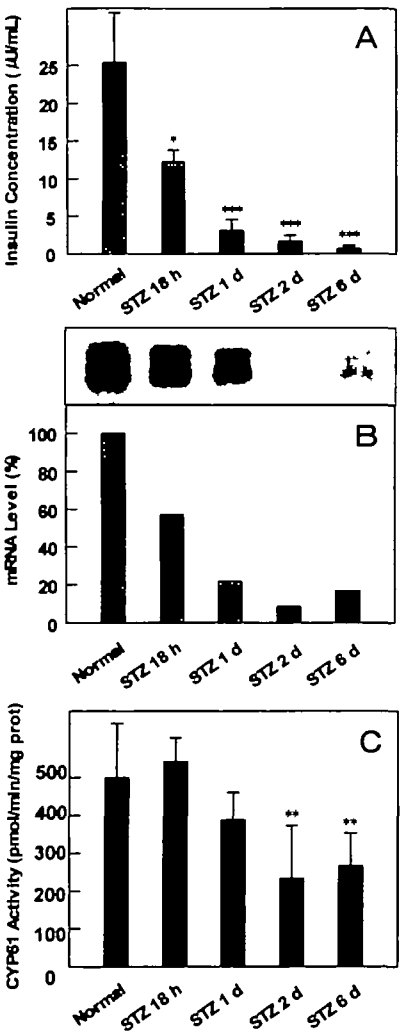

Fig 2 **Effects of STZ administration on the serum INS level and hepatic CYP51 mRNA and activity levels of the SD rats.** STZ (65 mg/kg of body weight) was subcutaneously injected into 24 rats. The rats were sacrificed 18 h (3 rats), 1 d (9 rats), 2 d (6 rats), and 6 d (6 rats) after the injection. Normal rats (9 rats) were untreated. The serum INS concentration and CYP51 activity were determined for individual animals. Northern blot analysis for determining CYP51 mRNA was made with the poly(A)$^+$ RNA specimen prepared from the combined RNA extract from the one group of rats. Since *CYP51* was transcribed into three different sizes (3.1, 2.7, and 2.3 kb) of mRNA in liver (*6*), the sum of their densities in the autoradiogram (panel B) was taken as the amount of CYP51 mRNA. Detailed procedures are described in "MATERIALS AND METHODS." A: Serum INS concentration (mean ± standard deviation; * and **, $p < 0.05$ and $p < 0.001$, respectively, relative to Normal). B Relative value of CYP51 mRNA level and autoradiogram C CYP51 activity (mean ± standard deviation; **, $p < 0.01$ relative to Normal).

pression. Hepatic CYP51 activity was also reduced by the STZ treatment (Fig 2C), although the extent of reduction was smaller than that of the mRNA level. This may be due to slow degradation of the active enzyme protein after the loss of CYP51 mRNA.

Both mRNA level and activity of hepatic CYP51 showed a slight increase 6 d after STZ treatment, although serum insulin remained at the lowest level (Fig. 2). This tendency suggests the possible presence of an additional inducing mechanism for *CYP51* that compensates for the decreased CYP51 level caused by the loss of serum insulin. However, these increments are not statistically significant, and this phenomenon has not been examired further.

Effect of INS Injection on Hepatic CYP51 Level—To obtain further evidence for the contribution of serum INS to the regulation of hepatic *CYP51* expression, the effect of INS administration on the STZ-diabetic SD rats was studied. Single subcutaneous injection of bovine INS (8 IU/kg of body weight) to the STZ-diabetic SD rats (6 d after STZ treatment, serum INS < 2 μIU/ml) caused rapid elevation of serum INS (> 320 μIU/ml) at 30 min after the injection. The increased serum INS returned nearly to the initial level (< 10 μIU/ml) within 3 h, and this low INS level was maintained 6 h after the injection. Although the peak level of serum INS reached more than 10 times the normal level (20–40 μIU/ml), no significant increment was observed in the hepatic CYP51 mRNA level and CYP51 activity at 3 and 6 h after the INS injection (data not shown). Significant increase of hepatic CYP51 mRNA was observed 12 h after the INS injection (Fig. 3). The serum INS level determined at this time was 5.7 ± 1.7 μIU/ml, which was about 2 to 3 times higher than that of the diabetic rats, and the mRNA level was significantly higher than that of the STZ-diabetic rats, although no significant increase of CYP51 activity was observed (Fig. 3). These observations suggested that hepatic *CYP51* might be induced slowly by serum INS. Then, the effects of repetitive administration of INS to the STZ-diabetic rats were examined.

Bovine INS (8 IU/kg of body weight) was injected 5 times at 3-h intervals into STZ-diabetic SD rats (6 d after STZ treatment, serum INS level < 2 μIU/ml), and the hepatic CYP51 mRNA level and CYP51 activity were determined 12 h after the last injection, *i.e.*, 24 h after the first INS injection. The serum INS level determined at this time was 13 ± 1.7 μIU/ml, which was about 40–50% of that of the normal rats and significantly higher than that of the STZ-induced diabetic rats. As shown in Fig. 3, marked elevation of the hepatic CYP51 mRNA level was observed after the repetitive administration of INS, and CYP51 activity was also restored nearly to the normal level.

These findings indicate that the induction of the hepatic *CYP51* gene required the continuous presence of a certain level of INS in serum, but onset of *CYP51* induction in response to serum INS might take place more than 6 h.

Hepatic CYP51 Expression under Various Serum INS Levels in Untreated Rats—To estimate the effect of physiologically high serum INS level on the hepatic *CYP51* expression, CYP51 mRNA level and CYP51 activity of WF rat, which is a model animal of INS-resistant diabetes showing high serum INS levels (*23*), and WL rat, the normal parent strain of WF, were examined. The serum INS level of WF rats used in this experiment was $2{,}972 \pm 450$ μIU/ml, which was about 30 times and more than 70 times higher than those of the normal WL and SD rats, respectively. However, the hepatic CYP51 activity and mRNA level of WF rats were considerably lower than those of WL rats (Fig. 4), and the CYP51 activity of the latter was comparable to that of the SD rats (Figs. 2 and 3). These facts suggest the contribution of INS receptor to the hepatic *CYP51* induction, because the INS tolerance of WF rat is

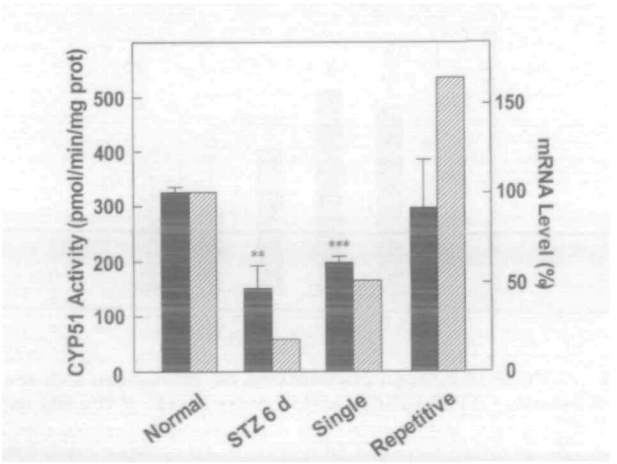

Fig. 3. **Effects of INS administration on hepatic CYP51 mRNA level and CYP51 activity in STZ-diabetic SD rats.** STZ-diabetic rats (6 d after STZ treatment described in Fig. 2) were injected subcutaneously with bovine INS (8 units/kg of body weight). The "repetitive" group was injected 5 times at 3-h intervals and sacrificed 12 h after the last injection. "Single" group was injected once and sacrificed 12 h after the injection. "Normal" and "STZ 6 d" represent untreated rats and rats 6 d after STZ treatment, respectively. Each group contained three or four rats. CYP51 mRNA level and CYP51 activity were assayed as described in the legend to Fig 2. Closed bars represent CYP51 activity (mean ± standard deviation; ** and ***, $p < 0.01$ and $p < 0.001$, respectively, to "Normal"), and hatched bars represent relative CYP51 mRNA level

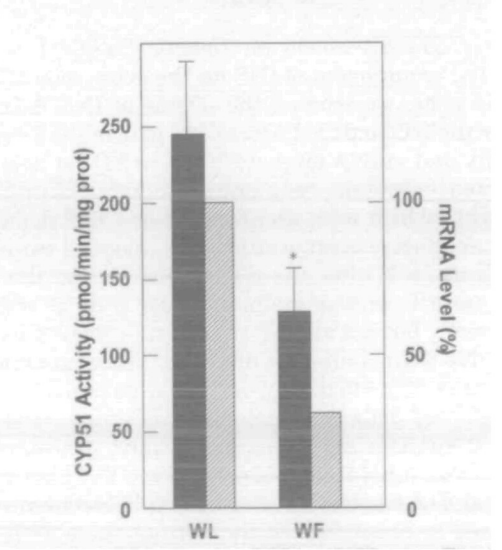

Fig 4 **Hepatic CYP51 levels of WF and WL rats.** Five frozen livers each of WF and WL rats provided by Dr Odaka of Takeda Chemical Industries were used. Microsomes and poly(A)$^+$ RNA were prepared as described in "MATERIALS AND METHODS" after thawing the frozen specimens. CYP51 mRNA level and CYP51 activity were assayed as described in the legend to Fig 2 Closed bars represent CYP51 activity (mean ± standard deviation, *, $p < 0.05$), and hatched bars represent relative CYP51 mRNA level.

reported to be due to impaired tyrosine kinase activity of INS receptor (*24*).

The serum INS level of WL rats (112 ± 23 μIU/ml) was more than twice of that of SD rats (Fig. 2). However, the hepatic CYP51 activity of WL rats (Fig. 4) was comparable to that of SD rats (Figs. 2 and 3). This observation suggests that the difference in serum INS level between SD and WL rats may not affect their hepatic CYP51 activity, and this assumption is supported by the fact that INS administration to normal SD rats caused no elevation of the hepatic CYP51 mRNA and activity level (data not shown). Ishida *et al.* (*11*) have reported that the expression level of hepatic CYP8B showed a circadian rhythm in response to the circadian variation of serum INS concentration. However, the hepatic CYP51 activity and CYP51 mRNA levels of the animals used in the previous experiments (*11*) remained constant throughout the day (data not shown). This fact indicates that the minimum concentration of serum INS in the circadian variation (*11*) maintained the steady-state level of hepatic CYP51. From these observations it can be concluded that the inductive effect of serum INS on the hepatic *CYP51* may be saturated at the concentration (20 to 40 μIU/ml) in normal SD rat serum.

Effect of STZ Administration on Testicular CYP51 Level—CYP51 is a single-copy housekeeping gene expressed ubiquitously (*5*, *6*). However, it has been reported that its expression in testis is somewhat different from that in other organs (*5*, *6*). For example, the CYP51 mRNA expressed in rat (*6*) and human (*5*) testis is considerably shorter (1.9 kb) than those expressed in other organs (3.1, 2.7, and 2 3 kb), and the transcription of *CYP51* gene into this short mRNA in rat testis has been reported to be regulated by CREMτ a cAMP-responsive element modulator (*9*). The effect of serum INS concentration on the expression of this short CYP51 mRNA and CYP51 activity in rat testis was examined. Figure 5 shows the level of the short CYP51 mRNA and CYP51 activity of testes obtained from the animals used in the experiment in Fig. 2. In the case of testicular CYP51, the level of 1.9 kb mRNA was not decreased but rather increased gradually by the STZ administration (Fig. 5). CYP51 activity was apparently reduced by STZ administration (Fig. 5), but this change was not statistically significant. These findings indicate that the expression of testicular *CYP51* does not depend on serum INS. In other words, the regulatory mechanism of testicular *CYP51* expression through the 1.9 kb mRNA (*5*, *6*) is different from that of the hepatic one.

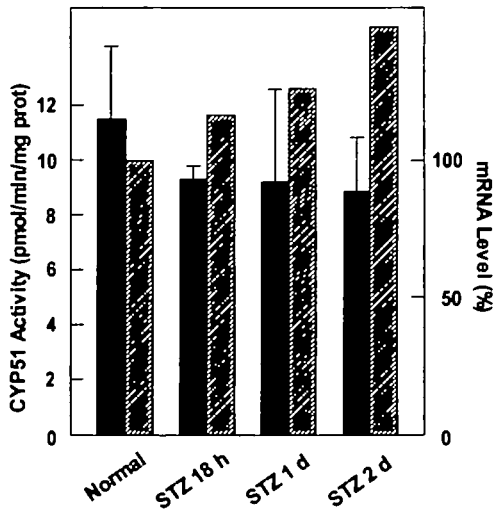

Fig. 5. **Effects of STZ administration on the testicular CYP51 mRNA level and CYP51 activity.** The testes used were isolated from the same animals as in the experiment in Fig. 2. The CYP51 activity was determined using the S-10 fraction as the enzyme source as described in "MATERIALS AND METHODS." Closed bars represent CYP51 activity (mean ± standard deviation), and hatched bars represent relative CYP51 mRNA level

DISCUSSION

Hepatic CYP51 mRNA level in rats was decreased in parallel with the decrement of serum INS concentration by STZ treatment (Fig. 2, A and B). Hepatic CYP51 activity was also reduced by the STZ treatment, although the reduction was delayed, possibly due to slower degradation rate of CYP51 protein (Fig. 2C). The decreased hepatic CYP51 mRNA level and CYP51 activity of the STZ-treated rats were restored within 24 h by the repetitive administration of INS (Fig. 3). It can be concluded from these observations that serum INS stimulates the expression of hepatic *CYP51* at the pretranslational level.

Hepatic CYP51 level remained constant throughout the day in spite of the clear circadian rhythm observed in serum INS concentration (*11*), and hepatic *CYP51* was not induced by INS administration to normal rats (see "RESULTS"). No significant difference was observed between the hepatic CYP51 activities of the normal SD and WL rats (Figs. 2, 3, and 4), although the serum INS concentration of the latter was more than twice that of the former. Consequently, it can be concluded that the minimum serum INS level observed in normal SD rats (20 to 40 μIU/ml) is high enough to support the induction of hepatic *CYP51*. In other words, the normal level of serum INS may support constitutive expression of hepatic *CYP51*. The hepatic CYP51 activity of the INS tolerant WF rats (Fig. 4) having a genetic defect in the tyrosine kinase linked to INS receptor (*24*) was considerably lower than those of the normal WL (Fig. 4) and SD rats (Figs. 2 and 3). This fact suggests the contribution of INS receptor to the INS-dependent expression of hepatic *CYP51*.

Significant induction of *CYP51* by INS administration to the STZ-treated rats was observed 12 h after the treatment, and repetitive administration of INS was necessary to restore the normal hepatic CYP51 activity and high mRNA level (Fig. 3). These facts indicate that the induction of hepatic *CYP51* does not respond quickly to an artificial surge of serum INS but requires the continuous presence of a certain level of INS in serum. Recently, Shimomura *et al.* (*25*) reported that the inductive effect of INS on hepatic lipid synthesis occurred through the induction of SREBP-1c, an isoform of sterol regulatory element binding proteins (SREBPs). This conclusion was derived from the following observations. The expression level of SREBP-1c was decreased in the liver of STZ-treated rats, and the reduced SREBP-1c level of the STZ-treated rats recovered to normal level 6 h after the INS administration (*25*). The effects of STZ and INS on hepatic CYP51 mRNA level discussed above were quite similar to their effects on hepatic SREBP-1c mRNA level except that the induction of *CYP51* required longer exposure to INS (Fig 3). These facts strongly sug-

gest that the elevation of hepatic *CYP51* expression level by INS may occur through the induction of the SREBP-1c gene. Sequence analysis of the promoter region of rat *CYP51* gene revealed the existence of a consensus sequence of sterol regulatory element (SRE)-1 core motif, CACCTCA, at 502 bp upstream of the initiating ATG codon (see the nucleotide sequence of rat *CYP51* exon-1; AB004087 in DDBJ/EMBL/GenBank), and Rozman *et al.* (*9*) reported that SREBP-1 bound to the corresponding SRE-1 of human *CYP51* gene. Consequently, it is highly likely that INS supports hepatic *CYP51* gene expression at the transcriptional level through the induction of SREBP-1c.

INS acts as an important factor for maintaining sterol synthesis in liver, as discussed above. On the other hand, INS is the potent suppressive factor of CYP8B and CYP7A, both of which are the essential enzymes of cholesterol catabolism to bile acids (*11*). Ishida *et al.* (*11*) pointed out the possibility that repression of the *CYP8B* gene by INS is effected by the insulin response sequence (IRS), which occurs in INS-repressive genes such as glucose 6-phosphatase (*26–28*), found in the promoter region of *CYP8B* gene. Consequently, INS is considered to act as an important regulatory factor elevating the hepatic cholesterol level by inducing its synthesis and repressing its catabolism.

INS did not affect the expression of testicular *CYP51* gene (Fig. 5). Torlinska *et al.* (*29*) suggested that rat testis is poor in INS receptor, based on the binding assay of INS to various rat organs, and Rozman *et al.* (*9*) reported that transcription of *CYP51* gene into the testis-specific 1.9 kb mRNA (*5*, *6*) in spermatids was regulated not by SREBP-1 but by CREMτ. The present result showing the insensitivity of testicular *CYP51* to serum INS level agrees well with these observations. Furthermore, we have reported that ovarian *CYP51* was induced by gonadotropin (*8*). These facts suggest that regulatory mechanism of *CYP51* gene expression in gonads is different from that in liver.

Taking these discussions together, we conclude that expression of hepatic *CYP51* is supported by a unified mechanism for enhancing hepatic lipid synthesis, which is driven by serum INS and SREBP-1c (*12*, *25*). Since liver is the principal organ of cholesterol synthesis and hepatic CYP51 dedicates this function, the inductive effect of INS described here must be the dominant regulatory mechanism of *CYP51* expression. The present work also found that the minimum concentration of INS observed in normal rats is sufficient for full induction of hepatic *CYP51*. Therefore, INS supports the constitutive expression of hepatic *CYP51* under normal physiological conditions. In contrast, gonadal *CYP51* expression is not dependent on INS. Different regulatory mechanisms of gonadal *CYP51* expression may relate to some special functions of gonadal CYP51, such as production of meiosis-activating sterols (*8*, *9*), which are the 14-demethylated metabolites of lanosterol (*7*). Consequently, further studies on organ-specific functions and relating regulatory mechanisms of *CYP51* are the important and interesting problems for considering functional diversification of this ubiquitously expressed and evolutionarily conserved P450.

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