Expression of the Peroxisome Proliferator-activated Receptor α
Gene Is Stimulated by Stress and Follows a Diurnal Rhythm*

(Received for publication, October 12, 1995)

Thomas Lemberger‡§, Régis Saladin§†, Manuel Vázquez§, Françoise Assimacopoulos**, Bart Staels‡¶, Béatrice Desvergne¶, Walter Wahlilt¶¶, and Johan Auwerx‡¶¶

From the Institut de Biologie Animale, Université de Lausanne, CH-1015 Lausanne, Switzerland, the Laboratoire de Biologie des Régulations chez les Eucaryotes, U.325 INSERM, Département d’Athérosclérose, Institut Pasteur, 59019 Lille, France, the **Département de Biochimie Médicale, Centre Médical Universitaire, CH-1211 Geneva, Switzerland

Peroxisome proliferator-activated receptors (PPARs) are nuclear hormone receptors that can be activated by fatty acids and peroxisome proliferators. The PPARα subtype mediates the pleiotropic effects of these activators in liver and regulates several target genes involved in fatty acid catabolism. In primary hepatocytes cultured in vitro, the PPARα gene is regulated at the transcriptional level by glucocorticoids. We investigated if this hormonal regulation also occurs in the whole animal in physiological situations leading to increased plasma corticosterone levels in rats. We show here that an immobilization stress is a potent and rapid stimulator of PPARα expression in liver but not in hippocampus. The injection of the synthetic glucocorticoid dexamethasone into adult rats produces a significant increase of PPARα expression in liver, whereas the administration of the antiglucocorticoid RU 486 inhibits the stress-dependent stimulation. We conclude that glucocorticoids are major mediators of the stress response. Consistent with this hormonal regulation, hepatic PPARα mRNA and protein levels follow a diurnal rhythm, which parallels that of circulating corticosterone. To test the effects of variations in PPARα expression on PPARα target gene activity, high glucocorticoid-dependent PPARα expression was mimicked in cultured primary hepatocytes. Under these conditions, hormonal stimulation of receptor expression synergizes with receptor activation by WY-14,643 to induce the expression of the PPARα target gene acyl-CoA oxidase. Together, these results show that regulation of the PPARα expression levels efficiently modulates PPAR activator signaling and thus may affect downstream metabolic pathways involved in lipid homeostasis.

The peroxisome proliferator-activated receptors (PPARs)

* This work was supported by the Etat de Vaud, the Swiss National Science Foundation, CNRS, INSERM, and by grants from the Fondation pour la Recherche Médicale (FRM) and the Association pour la Recherche sur le Cancer (ARC). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
† §§ Correspondence should be addressed either to W. Wahlil: Institut de Biologie Animale, Bâtiment de Biologie, Université de Lausanne, CH-1015 Lausanne, Switzerland. Tel.: 41-21-692-4110; Fax: 41-21-692-4105; E-mail: walter.wahlil@iba.unil.ch or to J. Auwerx, LBRE U135 INSERM, Institut Pasteur, 1 Rue Calmette, 59019 Lille Cédex, France. Tel.: 33-20-877-522; Fax: 33-20-877360.
‡§ Chargé de Recherche.
¶¶ Recipient of an European Science Foundation grant.
†† †‡ Chargé de Recherche.
††† †‡‡ Directeur de Recherche of the CNRS.
‡§§ Correspondence should be addressed either to W. Wahlil: Institut de Biologie Animale, Bâtiment de Biologie, Université de Lausanne, CH-1015 Lausanne, Switzerland. Tel.: 41-21-692-4110; Fax: 41-21-692-4105; E-mail: walter.wahlil@iba.unil.ch or to J. Auwerx, LBRE U135 INSERM, Institut Pasteur, 1 Rue Calmette, 59019 Lille Cédex, France. Tel.: 33-20-877-522; Fax: 33-20-877360.
¶¶ ¶ Directeur de Recherche of the CNRS.
††† †‡‡ Directeur de Recherche of the CNRS.
‡§§ Correspondence should be addressed either to W. Wahlil: Institut de Biologie Animale, Bâtiment de Biologie, Université de Lausanne, CH-1015 Lausanne, Switzerland. Tel.: 41-21-692-4110; Fax: 41-21-692-4105; E-mail: walter.wahlil@iba.unil.ch or to J. Auwerx, LBRE U135 INSERM, Institut Pasteur, 1 Rue Calmette, 59019 Lille Cédex, France. Tel.: 33-20-877-522; Fax: 33-20-877360.
in liver, including ACO and bifunctional enzyme, by peroxisome proliferators, suggesting that the level of expression of PPARα is important for the proper regulation of these genes in vivo (16). In fact, PPARα expression in adult rat liver is subject to marked individual variations for so far unknown reasons. \(^2\) We and others (28, 29) have shown recently that PPARα expression is directly regulated at the transcriptional level by glucocorticoids in rat hepatocytes or hepatoma cell lines cultured in vitro. This regulation is mediated by the glucocorticoid receptor and does not involve stabilization of the mRNA (28).

The expression of PPARα was increased by 3-fold in the plasma of rats injected with dexamethasone (28). The resulting increase in circulating glucocorticoid levels is important for the proper regulation of these genes (34, 35). The glucocorticoid levels in blood are elevated during the hypothalamic-pituitary-adrenal axis paradigm to study the regulation of a prototypical PPARα target gene, the ACO gene, is furthermore analyzed using hepatocytes in primary culture.

**Materials and Methods**

Animals and Treatment—Eight-week-old male Fisher 344 rats (BRL, Basel, Switzerland) were group-housed and had free access to water and food. The animals were kept on a 12-h light-dark cycle (light from 8:00 a.m. to 8:00 p.m.) in the stress and dexamethasone experiments; light from 7:30 a.m. to 7:30 p.m. in the diurnal variation experiment. All animals were accustomed to this cycle during at least 14 days. Rats were stressed by immobilization in transparent plastic tubes (5 cm diameter, 20 cm long) with small holes in the front allowing breathing and a hole at the back for the tail. Immobilization started at 9:00 a.m. The unstressed control animals were not manipulated during the duration of the experiment. Stressed (n = 3) and control (n = 3) rats were sacrificed at the same time. In the RU 486 experiments, both the stressed (n = 3) and control (n = 3) animals were injected subcutaneously with either vehicle or 30 mg/kg body weight (BW) of RU 486 (53) or 40 mg/kg body weight (BW) of RU 486 dissolved in 200 μl of propylene glycol. In the dexamethasone treatment experiments, animals were injected intraperitoneally under mild Forene (Abbott, Cham, Switzerland) anesthesia, with either vehicle (n = 3) or 40 μg/kg BW of dexamethasone dissolved in 500 μl of saline containing 1% ethanol (n = 3). In the diurnal variation experiment, the rats (12 animals) were sacrificed at precise time points (9:00 a.m., 3:30 p.m., 5:00 p.m., and 6:30 p.m.). For each time point, the animals (n = 3) were sacrificed on the three consecutive days. Since, in this experiment, the rats were housed in groups of five animals in four independent cages, the last animals killed were never alone, thus preventing an isolation stress. Furthermore, the rats were picked randomly among the four cages. The rats used in the dexamethasone and diurnal expression experiments were sacrificed by decapitation under Forene anesthesia, whereas rats in the stress experiments were sacrificed by exsanguination under ether anesthesia. Blood samples were collected in heparinized tubes (Millin, Geneva) and centrifuged. Plasma was frozen until analyzed. The tissues were rapidly dissected and frozen in liquid nitrogen.

**Corticosterone Measurements**—Plasma corticosterone levels were measured by radioimmunoassay (30).

RNA Preparation and Analysis—Total RNA was prepared using either the acid guanidinium thiocyanate-phenol-chloroform extraction method (31) or using the TRIzol Reagent (Life Technologies, Inc.). Quantification of PPARα and L27 mRNA levels by RNase protection assay were performed as described (28). ACO mRNA was detected by RNase protection using a rat ACO probe corresponding to the 447 nucleotide long SacI-SalI fragment of the full-length cDNA (32). Antisodies—A polyclonal antibody raised against the AB domain of mPPARα was generated as follows. A cDNA fragment corresponding to the 101 first amino acids of mPPARα was amplified by PCR from the full-length cDNA (Ref. 4, upstream primer: 5′-CCGGATCCATGGTG-CACAGGAGGC-3′, downstream primer: 5′-GGCGCTGTCAGGGCACCCTC-3′) and digested with BamH I and Smal. This fragment was cloned into the bacterial expression pQE-9 vector (Qiagen) using the BamH I and Klenow-filled HindIII sites. The same fragment was inserted into the pGEX-2T vector (Pharmacia Biotech Inc.) using the BamH I and Smal sites. The pQE-9 construct was used to overexpress the mPPARαAB domain fused to a 6×Histag in XL-1 bacteria (Stratagene). The resulting soluble polypeptide was purified from bacterial extract by affinity chromatography on a nickel-nitritotriacetic acid-agarose column under native conditions according to the manufacturer’s instructions (Qiagen, Hilden, Germany). The purified polypeptide was injected subcutaneously into KOBU rabbits (one primary and four booster injections of 200 μg). Serum was collected 10 days after the final antigen injection. To affinity purify the serum, an antigen-coupled column was prepared. The pGEX-2T construct described above was used to overexpress a GST-PPARα/AB domain fusion protein, which was purified onto a glutathione-Sepharose column (Pharmacia) and coupled to a N-hydroxysuccinimide-activated HiTrap column (Pharmacia). The resulting affinity column was then used to purify the immune serum. The resulting polyclonal antibody cross-reacts with rat PPARα, which is 98% conserved at the amino acid level in the AB region. Preimmune serum IgGs were purified using a Protein G-Sepharose 4 fast flow column (Pharmacia).

Nuclear extracts and Western Blotting—Nuclei were prepared as follows. Liver samples (0.5 g) or cultured hepatocytes (2.4 × 10⁶ cells) were homogenized in 0.5 M sucrose, 50 mM Tris-Cl, pH 7.5, 1 mM EDTA, 25 mM KCl (0.5 M sucrose TEKS). Cells were lysed with 0.5% Triton X-100 for 30 min at 4°C. The homogenate was then layered on a 0.9 M sucrose TEKS cushion and centrifuged at 2000 × g for 20 min. Nuclei were resuspended in 40% glycerol, 50 mM Tris-Cl, pH 8, 5 mM MgCl₂, and stored at −70°C. The concentrations of the nuclei were determined by measuring A at 260 nm in 5 M NaCl. Nuclei were lysed directly in SDS-gel loading buffer and loaded onto a 10% polyacrylamide-SDS gel. After electrophoresis on nitrocellulose, equal loading was checked by staining the blots with 0.2% Ponceau S red. The blots were blocked 1 h at 25°C with 5% non-fat dry milk in 25 mM Tris-Cl, pH 8.3, 140 mM NaCl, 2 mM KCl, and 0.05% Tween 20 (NFD TBS-Tween) and incubated overnight at 4°C with the primary antibody at a dilution of 1:1000 in 5% NFD TBS-Tween. Six 10-min washes at 25°C with 5% NFD TBS-Tween were performed. The filters were subsequently incubated 2 h at 25°C with the secondary antibody, horseradish peroxidase-conjugated goat anti-rabbit IgG (Cappel, Turnhout, Belgium), at a dilution of 1:1000 in 5% NFD TBS-Tween and washed nine times 10 min in TBS-Tween. Signal detection was achieved by chemiluminescence with the ECL system (Amersham) and 15- to 30-min exposure to X-ray film. Signals were quantified using an Ebscript 400/ATSM densitometer (ATH, Neuried, Switzerland).

Hepatocytes Primary Cultures—Rat hepatocytes were isolated by collagenase perfusion (33) of livers from 200–250 g rats (cell viability higher than 85% by trypan blue exclusion test). The hepatocytes were cultured in monolayer (1.5 × 10⁶ cells/cm²) in Williams’ E medium (Life Technologies, Inc.) supplemented with 5% fetal calf serum and antibiotics, at 37°C in a humidified atmosphere of 5% CO₂/95% air. Treatments with WY-14,643 (100 μM in ethanol) and dexamethasone (1 μM in ethanol) were started immediately after seeding.

**Results**

Stress Induces PPARα mRNA Expression—Physical and psychological stress triggers a multimodal response that mainly comprises the release of catecholamines by the sympathetic nervous system and glucocorticoids by the adrenal cortex (34, 35). The glucocorticoid levels in blood are elevated during experimental stress situations such as swimming, heat or cold exposure, photic or acoustic stimuli, and forced immobilization (36–38). Since the PPARα gene is under direct control of glucocorticoid hormones in rat hepatocytes cultured in vitro (28, 29), we used stress as an in vivo paradigm to study the regulatory effects of circulating glucocorticoids on the expression of the PPARα gene. In this study, an immobilization protocol was used because it is not associated with an increase of physical activity, in contrast to swimming for example. The reason to avoid experimental protocols requiring physical activity was that PPARs are involved in energy homeostasis (1). PPARα expression was analyzed in rats stressed by forced immobilization during 4 h. All stress experiments were started at 9 a.m. to circumvent interference with the diurnal variations of plasma corticosterone levels (see below). For the same reason, unstressed control animals were sacrificed at the same time as the stressed animals. The 4-h immobilization led to a 3-fold increase in the plasma levels of corticosterone, which is the

---

2 T. Lemberger, W. Wahl, and J. Auer, unpublished results.
major active glucocorticoid in rats (Fig. 1C). Total RNA was extracted from liver and hippocampus, a structure in the central nervous system that contains significant amounts of PPARα (39) and that has been described as one of the regions of the brain most sensitive to stress and glucocorticoids (40). Rat PPARα mRNA levels, as well as the levels of the mRNA of the large ribosomal subunit 27-kDa protein (L27) as a control, were assayed by RNase protection. After a 4-h immobilization, stressed animals displayed a 4.5-fold increase in the PPARα mRNA levels in liver relative to the unstressed animals (Fig. 1, A and B). Stress is therefore a potent physiological inducer of PPARα expression in liver. In contrast to the liver, no significant variation of PPARα mRNA levels could be detected in the hippocampus (Fig. 1, A and B).

To test whether glucocorticoids are indeed involved in the stress-dependent stimulation of PPARα expression, animals were treated before immobilization with the specific glucocorticoid antagonist RU 486 (30 mg/kg BW), or saline as control, and the effect on PPARα mRNA levels was analyzed. In the saline-injected animals, the 4-h immobilization produced a significant 3-fold increase of PPARα mRNA levels in liver (Fig. 2). In marked contrast, the stress-dependent induction of PPARα expression was inhibited in the rats injected with RU 486 (Fig. 2). These results demonstrate that glucocorticoids are the major endocrine mediators of the induction of PPARα expression by stress.

If glucocorticoids are indeed directly involved in the stimulation of PPARα mRNA expression induced by stress, the acute injection of exogenous glucocorticoids should lead to increased PPARα mRNA levels. Rats were hence injected either with saline or with dexamethasone (40 μg/kg BW) and sacrificed 4 h later. As predicted, dexamethasone-injected rats displayed 3.5-fold higher PPARα mRNA levels in liver as compared with saline-injected animals (Fig. 3, A and B). Thus, a single injection of the glucocorticoid agonist dexamethasone reproduced the effects of endogenous glucocorticoids secreted in response to stress. Another effect of administration of dexamethasone was the well described blockade of the hypothalamo-pituitary-adrenal axis (37), resulting in a dramatic decrease of the levels of circulating corticosterone (Fig. 3C).

Cycling of PPARα Expression—In rats, similar to the situation in other mammals, the circulating levels of glucocorticoids are subject to diurnal variations. The plasma levels of corticosterone, which are low in the morning, increase in the afternoon to reach a maximum about 2–3 h before the light-dark switch (41). In view of the results presented above, we expected the levels of PPARα mRNA to follow a similar diurnal rhythm. Thus, we compared PPARα mRNA expression in liver in the morning to its expression in the afternoon. The animals were kept on a 12-h light-dark cycle, with the light-dark switch at 7:30 p.m. Under these conditions, the peak of circulating corticosterone is expected to occur approximately at 5:00 p.m., which was indeed observed (Fig. 4A) Hence, liver samples were taken from animals sacrificed in the morning (9:30 a.m.) and at three different time points in the afternoon (3:30 p.m., 5:00 p.m., and 6:30 p.m.). The analysis was performed over three consecutive days to assess the periodic nature of the variations of PPARα expression and to exclude the possibility of an iso-

---

**Fig. 1. Stress stimulates PPARα expression in liver.** A 4-h immobilization stress was achieved as described under “Materials and Methods.” Total RNA (15 μg) was analyzed by RNase protection assay using a probe for the PPARα mRNA and a probe for the mRNA of the large ribosomal subunit 27-kDa protein (L27) as control. A, in the liver (left panel), the stressed animals (S, n = 3) show higher levels of PPARα mRNA than the unstressed control animals (C, n = 3). In contrast, there is no variation in PPARα mRNA levels in the hippocampus of the same animals (right panel). B, graphic representation of PPARα mRNA levels in liver and hippocampus of the control (C) and stressed (S) animals. PPARα mRNA levels were normalized to those of L27. The PPARα mRNA level in liver was arbitrarily set to 1. C, plasma corticosterone (CS) levels (ng/ml) of stressed (S) and control animals (C). Results are the mean ± S.D. of three animals.

**Fig. 2. RU 486 inhibits the stress-dependent stimulation of PPARα expression.** Rats were injected subcutaneously either with vehicle (SHAM) or with 30 mg/kg BW RU 486. Immediately after the injection, the control animals (C) were returned to their cage, whereas the stressed animals were subjected to a 4-h immobilization. Liver total RNA was analyzed as described in Fig. 1. A, representative results obtained after vehicle (SHAM) or RU 486 injection in control (C) and stressed (S) rats are shown. B, quantification of L27-normalized PPARα mRNA levels in liver of vehicle (SHAM) or RU 486 injected stressed (S) and control (C) animals. Results depict the mean ± S.D. of three animals.
lated stress having affected the animals. The mean values for PPARα mRNA levels determined for each of the time point are higher in the afternoon than at 9:30 a.m. (3.5-fold at 3:30 p.m.; 4-fold at 5:00 p.m.; 3-fold at 6:30 p.m., Fig. 4B). These variations of PPARα mRNA levels correlate well with the diurnal variations of plasma corticosterone levels (Fig. 4A), which strongly suggests that the PPARα gene responds to the diurnal variations of circulating corticosterone.

To test whether the diurnal variations of PPARα mRNA levels resulted into changes in PPARα protein expression, the relative levels of the receptor were measured in liver nuclear extracts. PPARα protein was detected on Western blots using an anti-PPARα antibody. This antibody detects a major band at 55 kDa, which corresponds to the predicted size of PPARα. This signal is specific, since it is not detected by preimmune IgGs (Fig. 4C, lane 1). Moreover, its intensity is markedly reduced when the antibody is co-incubated with 20 μg of the purified antigen (Fig. 4C, lane 6). In nuclear extracts from the liver of the animals analyzed during the second day of the 3-day experiment, PPARα protein levels were low in the morning and 2-, 3-, and 5-fold higher at 3:30 p.m., 5:00 p.m., and 6:30 p.m., respectively (Fig. 4C, lanes 2–5). Similar results were obtained for the two other days of the experiment. Thus, when the levels of PPARα mRNA and protein measured in each individual animal are plotted successively, according to the time at which the animal was sacrificed, both mRNA and protein levels show a striking cyclic pattern of expression over the three consecutive days (Fig. 4D).

Dexamethasone Potentiates the Induction of the ACO Gene by WY-14,643—Animal treatments by peroxisome proliferators, such as WY-14,643, produce multiple effects. Since specific PPAR antagonists have not yet been identified, it is difficult to distinguish between the PPAR-mediated direct effects of these hypolipidemic drugs and indirect effects involving metabolic or hormonal feedback mechanisms. Thus, we used hepatocytes in primary culture as an in vitro model to test whether the amount of PPARα protein is a limiting factor for the induction of its target genes. Dexamethasone provokes a 4-fold increase
Hormonal Regulation of PPARα Gene in Vivo

The hormonal response to stress involves essentially the release of catecholamines by the sympathetic nervous system and the secretion of glucocorticoids by the adrenal medulla through the activation of the hypothalamo-pituitary-adrenal axis (35). The onset and the duration of the glucocorticoid component of the stress response are slower and more sustained, respectively, than those of the catecholamine component. Thus, elevated glucocorticoid levels can be considered as a second hormonal wave following the initial peak of catecholamines. Our results demonstrate that, in vivo, the PPARα gene responds mainly to the glucocorticoid component of the hormonal response to stress. Indeed, using the antagonist RU 486 and the agonist dexamethasone, it was shown that glucocorticoids are necessary and sufficient to induce PPARα gene expression in liver during stress situations. Interestingly, stress was unable to modify PPARα expression in the hippocampus, despite the presence of glucocorticoid receptor in this tissue. One hypothesis is that liver-specific factors are required to permit the regulatory action of glucocorticoid receptor on the PPARα gene promoter. Alternatively, brain-specific factors might inhibit this regulation. In liver, the induction of PPARα mRNA is a fast response since it can be observed already 4 h after immobilization or agonist injection. The rapid regulation of PPARα mRNA expression in vivo reported herein argues for a similar direct transcriptional effect of glucocorticoids on PPARα gene expression in liver as in hepatocytes cultured in vitro (28, 29).

The metabolic response to stress is characterized by energy mobilization. Under the action of lipolytic hormones (catecholamines mainly), fatty acid mobilization occurs in the adipose tissue. In liver, the mobilized fatty acids enter the β-oxidation pathway and ketogenesis is stimulated (42). Remarkably, PPARα regulates genes involved in the activation of fatty acids as well as in the β-oxidation and ketogenesis pathways (1). Thus, stimulation of PPARα expression in liver by glucocorticoids during stress may potentiate the regulation of these target genes and contribute to the stimulation of the metabolic pathways involved in energy homeostasis.

The expression of the PPARα gene is showing a diurnal cycling pattern in liver, which parallels that of circulating corticosterone. This is consistent with a high sensitivity in liver of the PPARα gene to the levels of circulating corticosterone. The diurnal variations of PPARα mRNA is closely followed by a parallel cycling of PPARα protein suggesting that PPARα mRNA is efficiently translated. Furthermore, the cycling of the PPARα protein levels implies that the half-life of the protein is short enough to allow its levels to significantly decrease after 12 h. Altogether, these results suggest that PPARα signaling pathway may be efficiently modulated by a rapid and transient regulation of receptor levels. Post-translational mechanisms may furthermore exist, since we did not detect clear variations of PPARα protein levels after a 4-h stress (data not shown). Alternatively, this very short time of stimulation may be insufficient to give rise to a detectable increase of PPARα protein levels.

The investigation of the glucocorticoid-dependent regulation of PPARα gene was possible in the whole animal, since physiological situations associated with variations of circulating glucocorticoids are well characterized. Moreover, specific glucocorticoid agonists and antagonists are available. More difficult is the in vivo study of PPARα-mediated gene regulation, since the natural ligands of this receptor are still unknown, and specific agonists or antagonists have not been reported so far. The PPAR activators known to date, such as peroxisome proliferators, have pleiotropic effects in vivo, making it difficult to discriminate between the direct and indirect actions of these drugs in the animal. In contrast, hepatocyte primary cultures represent a model close to the in vivo situation, which is, however, isolated from the hormonal and metabolic complexity of the intact animal. Using this system, we show that high levels of expression of PPARα and activation of the receptor are necessary for a maximal stimulation of PPARα target genes. Indeed, the dexamethasone-dependent increase in PPARα expression is associated with a marked potentiation of the effects of the PPARα activator WY-14,643 on the expression of the ACO gene. These experiments provide evidence that the amount of receptor is a limiting factor and thus that the regulation of the level of PPARα expression by glucocorticoids has impact on the regulation of its target genes. In the whole animal, the exact physiological conditions in which such a glucocorticoid-dependent modulation of PPARα signaling occurs remain, however, to be investigated. For example, we did not observe diurnal variations in expression of the ACO gene (data not shown). This may well be due to the absence of significant levels of endogenous PPARα ligand. The same phe-
nomennon was observed in primary hepatocytes in which the induction of PPARα expression by dexamethasone is insufficient to stim-ulate ACO mRNA levels in the absence of an activator of the receptor. These observations suggest that activation of the receptor is a prerequisite for an effect of glucocorticoids on PPARα target genes. Investigation of physiological states in which these conditions are fulfilled, that is in which PPAR activators/ligand are produced, will possibly give important clues for the identification of the natural PPARα ligand.

In conclusion, data in this paper suggest that glucocorticoids have an important regulatory impact on PPARα expression in vivo. Physiological situations, such as stress and the diurnal surge of glucocorticoids, affect PPARα expression in liver. The regulation of PPARα expression provides a control mechanism which, when coupled to activator availability, regulates the PPARα action on its target genes and associated metabolic pathways.

Acknowledgments—We thank Roussel Uclaf, Romainville, France, for the gift of RU 486.

REFERENCES

1. Wahl, W., Braissant, O., and Desvergne, B. (1995) Chem. & Biol. 2, 261–266
2. Desvergne, B., and Wahl, W. (1995) in Inducible Transcription (Baeuerle, P., ed) Vol. 1, pp. 142–176, Birkhäuser, Boston.
3. Dreyer, C., Krey, G., Keller, H., Givel, F., Heffenbein, G., and Wahl, W. (1992) Cell 68, 879–887
4. Isseremann, I., and Green, S. (1990) Nature 347, 645–650
5. Göttlicher, M., Widmark, E., Li, Q., and Gustafsson, J. Å. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 7541–7545
6. Amri, E.-Z., Bonina, F., Allaud, G., Abumrad, N. A., and Grimaldi, P. A. (1995) J. Biol. Chem. 270, 2367–2371
7. Chen, F., Law, S. W., and O’Malley, B. W. (1993) Biochem. Biophys. Res. Commun. 196, 671–677
8. Kiiwetter, S. A., Forman, B. M., Blumberg, B., Ong, E. S., Borgmeyer, U., Mangelsdorf, D. J., Umeson, K., and Evans, R. M. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 7355–7359
9. Zhi, Y., Alvarado, K., Huang, Q., Rao, S. M., and Reddy, J. K. (1993) J. Biol. Chem. 268, 26817–26820
10. Tontonoz, P., Hu, E., Graves, R. A., Budavari, A. I., and Spiegelman, B. M. (1994) Genes & Dev. 8, 1224–1234
11. Sher, T., Yi, H. F., McBride, O. W., and Gonzalez, F. J. (1993) Biochemistry 32, 5598–5604
12. Greene, M. E., Blumberg, B., McBride, B., Yi, H. F., Kronquist, K., Kwan, K., Hsieh, L., Greene, G., and Nimer, S. D. (1995) Gene Expression 4, 281–299
13. Schmidt, A., Endo, N., Rutledge, S. J., Vogel, R., Shinar, D., and Rodan, G. A. (1992) Mol. Endocrinol. 6, 1634–1641
14. Dreyer, C., Keller, H., Mahfoudi, A., Lausten, V., Krey, G., and Wahl, W. (1993) Mol. Cell. Biol. 77, 67–76
15. Lock, E. A., Mitchell, A. M., and Elcombe, C. R. (1989) Annu. Rev. Pharmacol. Toxicol. 29, 145–163
16. Lee, S. S. T., Pineau, T., Drago, J., Lee, E. J., Owens, J. W., Krotz, D. L., Fernandez-Salgueiro, P. M., Westphal, H., and Gonzalez, F. J. (1995) Mol. Cell. Biol. 15, 3012–3022
17. Schoonjans, K., Watanabe, M., Suzuki, H., Mahfoudi, A., Krey, G., Wahl, W., Grimaldi, P., Staels, B., Yamamoto, T., and Auwerx, J. (1995) J. Biol. Chem. 270, 19269–19276
18. Keller, H., Dreyer, C., Medin, J., Mahfoudi, A., Ozato, K., and Wahl, W. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 2166–2164
19. Tugwood, J. D., Issemann, I., Anderson, R. G., Bundell, K. R., McPheat, W. L., and Green, S. (1992) EMBO J. 11, 433–439
20. Zhang, B., Marcus, S. L., Sajjadi, F. G., Alvares, K., Reddy, J. K., Subramani, S., Rachubinski, R. A., and Capone, J. P. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 7541–7545
21. Gulik, T., Cresci, S., Caira, T., Moore, D. D., and Kelly, D. P. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 11012–11016
22. Muehoffer, A. S., Griffin, K. J., and Johnson, E. F. (1992) J. Biol. Chem. 267, 19051–19053
23. Rodriguez, J. C., Gil-Gomez, G., Hegardt, F. G., and Hado, D. (1994) J. Biol. Chem. 269, 18767–18772
24. Vu-Dac, N., Schoonjans, K., Laine, B., Fruchart, J.-C., Auwerx, J., and Staels, B. (1994) J. Biol. Chem. 269, 30112–30118
25. Vu-Dac, N., Schoonjans, K., Ksaykh, V., Dallongeville, J., Fruchart, J.-C., Staels, B., and Auwerx, J. (1995) J. Clin. Invest. 95, 741–750
26. Hertz, R., Bishara-Shieban, J., and Bar-Tana, J. (1995) J. Biol. Chem. 270, 13470–13475
27. Lehmann, J. M., Moore, L. B., Smith- Oliver, T. A., Wilkinson, W. O., Willson, T. M., and Kiiwetter, S. A. (1995) J. Biol. Chem. 270, 12953–12956
28. Lemberger, T., Staels, B., Saladin, R., Desvergne, B., Auwerx, J., and Wahl, W. (1994) J. Biol. Chem. 269, 24527–24530
29. Steiniger, H. H., Sørensen, H. N., Tugwood, J., Skrede, S., Spydevold, Ø., and Gautvik, K. M. (1994) Eur. J. Biochem. 225, 967–974
30. Grassow-Cohen, A., Chen, C. L., and Besch, E. L. (1982) Proc. Soc. Exp. Biol. Med. 170, 29–34
31. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
32. Miyazawa, S., Osumi, T., Hashimoto, T., Ohno, K., Miura, S., and Fujiki, Y. (1989) Mol. Cel. Biol. 9, 83–91
33. Berry, M. N., and Friend, D. S. (1969) J. Cell Biol. 43, 506–520
34. Axelrod, J., and Reisine, T. D. (1984) Science 224, 452–459
35. McEwen, B. S., and Sapolsky, R. M. (1995) Curr. Opin. Neurobiol. 5, 205–216
36. Alexandrova, M., and Farkas, P. (1992) J. Steroid Biochem. Mol. Biol. 42, 483–488
37. Weidner, D., and Feldman, S. (1993) Neuroendocrinology 58, 49–56
38. Guillaume-Gentili, C., Rohner-Jeanrenaud, F., Abram, F., Besstetti, G. E., Ross, G. I., and Jeanrenaud, B. (1990) Endocrinology 126, 1875–1879
39. Braissant, O., Fouillée, F., Scatena, C., Dauca, M., Wahl, W. (1996) Endocrinology, in press
40. McEwen, B. S. (1991) La Presse Médicale 20, 1901–1906
41. Dhabhar, F. S., McEwen, B. S., and Spencer, R. L. (1993) Brain Res. 616, 89–98
42. Neeveshime, E. A., and Start, C. (eds) (1973) Regulation in Metabolism, pp. 293–323, John Wiley & Sons, New York

Hormonal Regulation of PPARα Gene in Vivo

1769