The Glucocorticoid-responsive Gene Cascade

ACTIVATION OF THE RAT ARGINASE GENE THROUGH INDUCTION OF C/EBPβ

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The gene for liver-type arginase, an ornithine cycle enzyme, is induced by glucocorticoids in a delayed secondary manner. An enhancer element located around intron 7 of the rat arginase gene shows delayed glucocorticoid responsiveness, and it harbors two sites binding with members of the CCAAT/enhancer binding protein (C/EBP) family. Here, we investigate the role of these C/EBP binding sites in glucocorticoid response of the arginase gene. When inserted in front of the herpes simplex virus thymidine kinase promoter, these C/EBP sites exhibited glucocorticoid responsiveness in reporter transfection assay using rat hepatoma H4IE cells. In footprint analysis using nuclear extracts of H4IE cells, profiles of the protected areas of the two C/EBP sites changed when cells were treated with dexamethasone. In gel shift analysis, the complex formation for the two C/EBP sites was augmented in response to dexamethasone. Antibody supershift/inhibition analysis demonstrated that a major portion of the binding proteins induced by dexamethasone is C/EBPβ. Induction of arginase mRNA by dexamethasone was preceded by augmentation of the C/EBP site-binding activities, which followed increase in C/EBPβ mRNA. These results were consistent with the notion that the glucocorticoid response of the arginase gene is mediated by C/EBPβ.

Liver-type arginase (EC 3.5.3.1) is an enzyme catalyzing the last step of the ornithine cycle (urea cycle), through which toxic ammonia is converted into less toxic urea. The mammalian arginase gene is expressed almost exclusively in the liver and is markedly induced in the late fetal period (3). After birth, arginase expression is also activated by high protein intake or starvation that imposes ammonia production (4, 5). These developmental and nutritional activations of the arginase gene are presumably mediated, at least in part, by glucocorticoids and/or glucagon. In fact, these hormones induce arginase mRNA in rat primary cultured hepatocytes (6) and a rat hepatoma cell line H4IE cell (7).

Glucocorticoids activate transcription of many liver-specific genes. The glucocorticoid response can be divided into two types. One is the primary response, in which the glucocorticoid-receptor complex directly activates transcription of target genes and which does not require ongoing protein synthesis. The other is the secondary response that follows a delayed time course, compared with the primary response, and is blocked by protein synthesis inhibitors. Genes showing the secondary response are exemplified by those of hepatic α2u-globulin (8), its isofrom (9, 10), and ornithine cycle enzymes including arginase (6). A newly synthesized protein factor(s) involved in this secondary activation process has remained to be identified.

We have studied transcriptional regulation of the arginase gene by investigating the promoter (11–13) and the enhancer (12) of the rat gene (14). The arginase promoter exhibits a moderate liver-selective activity (11) but apparently no glucocorticoid responsiveness (12). On the other hand, the enhancer region, which is located 11 kilobases downstream from the transcription start site and spans the junction of intron 7 and exon 8, showed delayed glucocorticoid responsiveness in transfection analysis using rat hepatoma H4IE cells (12). In this enhancer region, there are four protein binding sites, two of which are recognized by a factor(s) related to CCAAT/enhancer binding protein (C/EBP)1 (15) and the other two bind with unknown factors. C/EBP is characterized by the DNA-binding basic region and the adjacent leucine zipper domain (16) and constitutes a family with related factors (15, 17). C/EBPβ is one of these family members (16) and is also known as NF-IL6 (18), IL6-DPB (19), LAP (20), AGP/EBP (21), and CRP2 (17). When C/EBPβ is enriched in the liver and plays a role in liver-selective transcription of several genes (20, 22, 23), this factor seems to be involved in a number of cellular processes in various tissues (reviewed in Introductions of Refs. 24–27). In hepatoma cells (28, 29) and primary-cultured rat hepatocytes (30), C/EBPβ mRNA is induced by glucocorticoids. This induction in hepatocytes is caused by transcriptional activation of the C/EBPβ gene in a primary manner (30).

Here, we asked whether the C/EBP sites in the arginase enhancer mediate the glucocorticoid response. We also examined changes in profiles of protein binding to these sites in response to glucocorticoids. Our findings suggest that C/EBPβ is involved in the secondary glucocorticoid response of the arginase gene.

MATERIALS AND METHODS

Plasmids—The basal chloramphenicol acetyltransferase (CAT) plasmid pBLCAT5-ABA was constructed starting from the plasmid pBLCAT5 (31) that harbors the CAT gene under the control of herpes simplex virus thymidine kinase gene (tk) promoter. pBLCAT5 was cut with BglII, blunt-ended with Klenow fragment, and self-ligated. The resulting plasmid was cut with SacI, and the linker 5′-CGGGCCCAGATCTGGGCCCGAGCT-3′ bearing the ApoI-BglII-ApoI sites was inserted into the SacI site, yielding the plasmid pBLCAT5-ABA. Oligonucleotides each containing C/EBP binding sites A and B (previously designated protein-binding sites I and III (12), respectively) of the arginase enhancer were inserted into the BamHI site located just

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1 The abbreviations used are: C/EBP, CCAAT/enhancer binding protein; CAT, chloramphenicol acetyltransferase; tk, thymidine kinase gene; MBP, maltose-binding protein.
upstream of the tk promoter of pBLCAT5-ABA. The direction and sequence integrity of the inserts were verified by nucleotide sequencing.

CAT Assays—Rat hepatoma H4IIE cells were grown in Eagle’s minimal essential medium supplemented with 10% fetal calf serum. Transfection was carried out by the calcium phosphate precipitation method (32), using a total of 15 μg of DNA mixture containing 10 μg of the C/EBPα recombinant plasmid and 5 μg of an internal standard plasmid pAe-lacZ (33) bearing the β-galactosidase gene. Cells were cultured with or without 1 μM dexamethasone for 48 h prior to harvesting. During this period, 1 mM dibutyryl cAMP was also added into the medium to increase the basal CAT activity (12). 72 h after transfection, CAT activity of cell extracts was measured as described (34), quantified using a bio-image analyzer BAS2000 (Fuji Photo Film, Tokyo), and normalized for β-galactosidase activity. Relative CAT activities were shown by mean values plus standard errors of at least three independent experiments.

DNase I Footprint Analysis—Nuclear extracts from rat liver (35) and H4IIE cells (36) were prepared as described. The probe used was the 32P-labeled DNA fragment corresponding to the lower strand of the XbaI-HincII enhancer segment (12). The binding reaction, DNase I digestion, and electrophoresis were done as described previously (12).

Gel Shift Assays—Expression and purification of the recombinant C/EBPβ protein fused to maltose-binding protein (MBP) was as described (13). Double-stranded oligonucleotide probes were 5′-end labeled with [γ-32P]ATP and T4 polynucleotide kinase. The binding reaction for 30 min on ice was carried out as described previously (12). Electrophoresis was done in a 5% polyacrylamide gel made in 22 mM Tris, 22 mM boric acid, and 0.6 mM EDTA. The antibodies used in supershift/inhibition analysis (1 μg of IgG in 1 μl) against C/EBPa and C/EBPβ were purchased from Santa Cruz Biotechnology, Inc.

RNA Blot Analysis—Isolation of total RNA (37), electrophoresis, blotting, and hybridization (30) were performed as described. The following 32P-labeled DNA probes were used: the mouse C/EBPβ gene, about a 1.6-kilobase BstXI fragment of pEF-C/EBPβ (22); rat arginase cDNA, about a 850-base pair EcoRI-EcoRV fragment of p ARG-2 (38); and rat β actin, a polymerase chain reaction product, nucleotide positions 417–1223 (39). Relative mRNA levels quantified with a bio-image analyzer were shown by mean values with standard errors of at least three independent experiments.

RESULTS

Mediation of Glucocorticoid Response by C/EBP Binding Sites of the Arginase Enhancer—The arginase enhancer, which shows glucocorticoid responsiveness in a delayed secondary manner, contains four protein binding sites (12). Two sites (sites A and B) bind C/EBP family members (Fig. 1). To examine the contribution of the two C/EBP binding sites to glucocorticoid responsiveness, we inserted each C/EBP binding site just in front of the herpes simplex virus tk promoter that drives the Escherichia coli CAT gene, and transient transfection assay was done using rat hepatoma H4IIE cells. The cells were treated with 1 μM dexamethasone for 48 h, and cell extracts were subjected to CAT enzyme assay (Fig. 1). CAT activity derived from the native tk promoter was increased slightly (1.8-fold) by the dexamethasone treatment. When site A was inserted in the same direction of the tk promoter, CAT activity was enhanced 13.6-fold in the absence of dexamethasone and was not further augmented by dexamethasone. On the other hand, when inserted in the opposite direction, site A enhanced binding of CAT activity 4.4-fold, and this enhanced activity was further augmented 4.2-fold by dexamethasone. Therefore, site A can respond to dexamethasone if appropriately arranged with the tk promoter. The reason why site A does not respond to dexamethasone in the forward direction is not known. A possible explanation is that in this promoter construct an unidentified transcriptional activator(s) occupies site A and is insensitive to dexamethasone. Another C/EBP binding site B had little effect on basal CAT activity when a single copy of this site was inserted in forward or reverse direction. On the other hand, CAT activities derived from these constructs increased 5.3- and 3.9-fold, respectively, in response to dexamethasone. Two copies of site B inserted in the forward direction led to a 5.3-fold increase in the basal tk promoter activity, which was further augmented 4.2-fold by dexamethasone. Therefore, site B exhibits glucocorticoid responsiveness in the context of the tk promoter. Thus, both C/EBP binding sites A and B have potency to mediate the glucocorticoid response.

Changes in Footprint Profiles of the C/EBP Binding Sites by Dexamethasone—To examine whether dexamethasone affects protein-binding profiles of the C/EBP sites, DNase I footprint analysis was carried out (Fig. 2). Using nuclear extracts from H4IIE cells, protection against DNase I digestion was observed at C/EBP sites A and B corresponding to footprint areas previously detected with rat liver nuclear extracts (12). Treatment of H4IIE cells with 1 μM dexamethasone for 12 h changed the footprint profiles of both sites A and B. In both sites, the footprint profiles obtained with extracts from dexamethasone-treated cells resembled those seen with liver extracts. Therefore, binding activity (or activities) to C/EBP sites A and B similar to that in the liver seems to be induced in H4IIE cells by dexamethasone.

Induction of C/EBPβ Binding to Sites A and B by Dexamethasone—We further characterized dexamethasone-induced changes in a factor(s) binding to sites A and B with gel mobility shift assay (Fig. 3A). We previously showed that C/EBPα and C/EBPβ bind with these sites and that C/EBPα dominates in the rat liver, whereas C/EBPβ dominates in the dexamethasone-treated H4IIE cells (12). When H4IIE cells were treated with 1 μM dexamethasone for 12 h, binding of nuclear extracts to sites A and B was dramatically enhanced (compare lanes 5 to lanes 1). To examine whether C/EBPβ is involved in dexamethasone-induced binding activities, antibody supershift/inhibition analysis was performed. The antibody specific to

![Figure 1. Glucocorticoid responsiveness of C/EBP sites in the arginase enhancer region.](image1)

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C/EBPβ diminished the complexes induced by dexamethasone (lanes 7), while the C/EBPα-specific antibody and the control serum caused little change (lanes 6 and 8). Therefore, C/EBPβ accounts for a major portion of site A- and B-binding activities induced by dexamethasone in H4IIE cells. Similar results were obtained by using as a probe the authentic C/EBP site (40) of the Rous sarcoma virus long terminal repeat (data not shown).

To confirm recognition of sites A and B by C/EBPβ, we examined binding of the recombinant C/EBPβ protein fused to MBP (Fig. 3B). When sites A and B, as well as the authentic Rous sarcoma virus C/EBP site, were used as probes, the MBP-C/EBPβ fusion protein but not MBP gave a shifted band. On the other hand, no shifted band was detected when an unrelated probe (site IV (12) of the arginase enhancer) was used. Thus, sites A and B specifically bind with the recombinant C/EBPβ protein. All these results taken together lead to the proposal that binding activity for C/EBP sites A and B of the arginase enhancer is induced in H4IIE cells by dexamethasone administration and that this binding can be attributable to C/EBPβ.

Serial Inductions of C/EBPβ mRNA, C/EBP Site-binding Activity, and Arginase mRNA—Based on the observations described above and the primary glucocorticoid response of the C/EBPβ gene (30) and the secondary response of the arginase gene (6), we hypothesized that C/EBPβ induced primarily by dexamethasone is responsible for the secondary induction of the arginase gene. We then examined whether time courses for increases in C/EBPβ mRNA level, C/EBP site-binding activity, and arginase mRNA level by dexamethasone are concordant with this hypothesis. In Fig. 4A, RNA blot analysis was carried out after exposure of H4IIE cells to dexamethasone for various periods. Arginase mRNA began to increase with a lag of 6 h following the hormone addition and increased markedly at 24 h. On the other hand, C/EBPβ mRNA began to increase as early as 0.5 h and reached a plateau at 2 h. β-actin mRNA, measured as a standard, was practically unchanged. Time courses of changes in binding activities for C/EBP sites A and B were also monitored (Fig. 4B). For both sites A and B, binding activities were increased markedly 6 h after the addition of dexamethasone and were sustained by 24 h. No obvious change was observed for NF-I site-binding activity monitored as a standard. These results were quantified in Fig. 4C. Concordant with our hypothesis, the increase in arginase mRNA was pre-

FIG. 2. Changes in footprint profiles of C/EBP sites A and B of the arginase enhancer region by dexamethasone. The lower strand of the arginase enhancer fragment was subjected to DNase I footprint analysis using nuclear extracts from the rat liver or H4IIE cells cultured with (+) or without (−) 1 μM dexamethasone (Dex) for 12 h. Lanes A–C show the chemical cleavage of the probe as sequence markers. Lanes extract (−) show the control digestion pattern obtained without nuclear extracts. Boxes marked A and B alongside the autoradiograms represent areas protected from DNase I digestion.

FIG. 3. C/EBPβ binding activity induced by dexamethasone. A, dexamethasone-induced changes in gel shift patterns and antibody supershift/inhibition analysis. H4IIE cells were treated (+) or not treated (−) with 1 μM dexamethasone (Dex) for 12 h, and nuclear extracts were prepared. Probes for C/EBP sites A and B (Fig. 1) of the arginase enhancer were incubated with the nuclear extracts (5 μg of protein) from H4IIE cells, and gel shift assay was performed as described under "Materials and Methods." Antibody (Ab) against C/EBPα or C/EBPβ or control nonimmune serum was added to the binding mixture halfway through the reaction. The bracket shows specific complexes that can be competitively inhibited by the homologous oligonucleotide (data not shown). The asterisk indicates a nonspecific band. B, binding of the MBP-C/EBPβ fusion protein to the arginase enhancer sites. Probes for the authentic C/EBP binding site of the Rous sarcoma virus long terminal repeat (40), sites A and B of the arginase enhancer, and an unrelated site (protein binding site IV (12) of the arginase enhancer) were incubated with bacterially expressed MBP or the MBP-C/EBPβ fusion protein (10 ng each), and gel shift assay was performed.
ceded by the increases in C/EBP site-binding activities, which were further preceded by the increase in C/EBPβ mRNA.

DISCUSSION

In this report, we presented evidence supporting the notion that C/EBPβ mediates the secondary glucocorticoid response of the arginase gene. First, C/EBP sites of the arginase enhancer, when linked to the heterologous tk promoter, exhibited glucocorticoid responsiveness in reporter transfection assay. Second, protein binding profiles of these C/EBP sites were changed by dexamethasone, and C/EBPβ occupied the major portion of newly induced binding activities. Third, induction of arginase mRNA by dexamethasone followed an increase in C/EBP site-binding activity that was preceded by C/EBPβ mRNA induction. We previously showed that C/EBPβ mRNA is induced by dexamethasone through transcriptional activation without requiring de novo protein synthesis (30). On the other hand, the induction of arginase mRNA follows a delayed time course and requires ongoing protein synthesis (6, 30). Based on these observations, we propose the following: glucocorticoids primarily activate the C/EBPβ gene and the induced C/EBPβ secondarily activates the arginase gene by binding to the enhancer region.

In cotransfection assay using non-hepatic Chinese hamster ovary cells, CAT activity derived from the reporter construct containing one copy of site B in the forward direction was activated weakly (1.7-fold) by a C/EBPβ expression plasmid. It remains to be clarified whether an additional factor(s) is required for the maximal action of dexamethasone.

In general, gene cascades controlled by steroid hormones underlie various biological processes. A well-documented example is effects of ecdysone in metamorphosis of Drosophila. Ecdysone triggers induction of a relatively small number of primary response genes, some of which encode transcription factors. These induced factors in turn activate more than 100 secondary response genes (reviewed in Ref. 41). In mammals, several transcription factors, as well as C/EBPβ, were shown to be primarily induced by glucocorticoids in various tissues: C/EBPβ in adipocytes (42), peroxisome proliferator-activated

3 T. Gotoh, S. Chowdhury, M. Takiguchi, and M. Mori, unpublished results.
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receptor (PPAR) α in hepatocytes (43), and IxB in immune cells and Hela cells (44, 45). On the other hand, especially in the liver, glucocorticoid induction of a number of genes exhibits more or less secondary aspects, i.e. delayed time course and/or sensitivity to protein synthesis inhibitors, as exemplified by genes for α2u-globulin (8–10), α1-acid glycoprotein (28, 46), albumin (47), tryptophan oxygenase (48), phosphoenolpyruvate carboxykinase (49), and ornithine cycle enzymes (6). In mammals, little is known about the transcription factors responsible for secondary activation of target genes. Involvement of C/EBP β in glucocorticoid response of the arginase gene provides a typical example for hierarchical gene regulation by glucocorticoids.

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REFERENCES

1. Herzfeld, A., and Raper, S. M. (1976) Biochem. J. 153, 469–478
2. Kawamoto, S., Amaya, Y., Oda, T., Kuzumi, T., Saheki, T., Kimura, S., and Mori, M. (1986) Biochem. Biophys. Res. Commun. 136, 955–961
3. Morris, S. M. Jr., Moncane, C. L., Rand, K. D., Dizikes, G. J., Cederbaum, S. D., and O’Brien, W. E. (1987) Arch. Biochem. Biophys. 256, 343–353
4. Schimke, R. T. (1992) J. Biol. Chem. 267, 459–468
5. Schimke, R. T. (1962) J. Biol. Chem. 237, 1921–1924
6. Nebes, V. L., and Morris, S. M., Jr. (1988) Mol. Endocrinol. 2, 444–451
7. Dizikes, G. J., Spector, E. B., and Cederbaum, S. D. (1986) Somat. Cell Mol. Genet. 12, 375–384
8. Chen, C.-L. C., and Feigelson, P. (1980) Ann. N. Y. Acad. Sci. 349, 28–45
9. Addison, W. R., and Kurtz, D. T. (1986) Mol. Cell. Biol. 6, 2334–2546
10. Hess, P., and Payvar, F. (1992) J. Biol. Chem. 267, 3490–3497
11. Takiguchi, M., and Mori, M. (1991) J. Biol. Chem. 266, 9186–9193
12. Gotoh, T., Haraguchi, Y., Takiguchi, M., and Mori, M. (1994) J. Biochem. (Tokyo) 115, 778–788
13. Chowdhury, S., Gotot, T., Mori, M., and Takiguchi, M. (1996) Eur. J. Biochem. 236, 500–509
14. Ohtake, A., Takiguchi, M., Shigeto, Y., Amaya, Y., Kawamoto, S., and Mori, M. (1988) J. Biol. Chem. 263, 2245–2249
15. Cao, Z., Umek, R. M., and McKnight, S. L. (1991) Genes & Dev. 5, 1538–1552
16. Landschulz, W. H., Johnson, P. F., Adashi, E. Y., Graves, B. J., and McKnight, S. L. (1988) Genes & Dev. 2, 786–800
17. Williams, S. C., Cantwell, C. A., and Johnson, P. F. (1991) Genes & Dev. 5, 1553–1567
18. Akira, S., Ishihoki, H., Sugita, T., Tanabe, O., Kinoshita, S., Nishio, Y., Nakajima, T., Hirano, T., and Kishimoto, T. (1990) EMBO J. 9, 1897–1906
19. Poli, V., Mancini, F. P., and Cortese, R. (1990) Cell 63, 643–653
20. Descombes, P., Chalpier, M., Lichtsteiner, S., Falvey, E., and Schibler, U. (1999) Genes & Dev. 13, 1541–1551
21. Chang, C.-J., Chen, T.-T., Lei, H.-Y., Chen, D.-S., and Lee, S.-C. (1999) Mol. Cell. Biol. 10, 6642–6653
22. Nishiyori, A., Tashiro, H., Kimura, A., Akagi, K., Yamamura, K., Mori, M., and Takiguchi, M. (1994) J. Biol. Chem. 269, 1323–1331
23. van Ooij, C., Snyder, R. C., Paaper, B. W., and Dueser, G. (1992) Mol. Cell. Biol. 12, 3023–3031
24. Yeh, W.-C., Cao, Z., Classon, M., and McKnight, S. L. (1995) Genes & Dev. 9, 168–181
25. Tanaka, T., Akira, S., Yoshida, K., Umemoto, M., Yoneda, Y., Shirafuji, N., Fujiiwara, H., Stuematsu, S., Yoshida, N., and Kishimoto, T. (1995) Cell 80, 353–361
26. Screpanti, I., Romani, L., Musiani, P., Modesti, A., Fattori, E., Lazzaro, D., Selliuto, C., Scarpinato, S., Bellavita, D., Lattanzio, G., Bistoni, F., Frati, L., Cortese, R., Gulino, A., Ciliberto, G., Costantini, F., and Poli, V. (1995) EMBO J. 14, 1932–1941
27. Nishio, Y., Ishihoki, H., Kishimoto, T., and Akira, S. (1993) Mol. Cell. Biol. 13, 1854–1862
28. Baumann, H., Haller, G. P., Morella, K. W., Kruse, S. J., and Mosser, M. K. (1991) J. Biol. Chem. 266, 20390–20399
29. Baumann, H., Morella, K. K., Campos, S. F., Cao, Z., and Jarrett, G. P. (1992) J. Biol. Chem. 267, 19744–19751
30. Matsuno, F., Chowdhury, S., Gotot, T., Iwase, K., Matsuzaki, H., Takatsuki, K., Mori, M., and Takiguchi, M. (1996) J. Biochem. (Tokyo) 119, 524–532