THE ROLE OF C/EBPα IN THE ACTION OF TCDD TO CAUSE SHIFTS IN CELLULAR ENERGY METABOLISM

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Introduction

Regulation of energy metabolism in adipose tissue and liver requires the coordinated expression of genes encoding lipogenic and gluconeogenic enzymes in response to hormonal stimuli. The molecular mechanisms by which these complex events occur have only recently begun to be elucidated. One putative regulator of lipogenesis and gluconeogensis is the CCAAT/enhancer binding protein (C/EBPα) a transcription factor containing a basic leucine zipper motif[1]. A close relationship between C/EBPα and energy homeostasis has been hypothesized based on several lines of biochemical and genetic evidence[2]. C/EBPα has been shown to *trans*-activate several genes, including stearoyl-CoA desaturase 1, GLUT4, FABP, and acetyl CoA carboxylase, involved in the uptake and synthesis of metabolic fuels[3-5]. Expression of C/EBPα mRNA is highest in adipose, liver and intestine, tissues involved in metabolic fuel uptake, synthesis and storage and is dramatically increased just prior to birth[6]. Finally, analysis of mice that had the *c/epbα* gene deleted has provided compelling evidence for a link between C/EBPα and energy metabolism. The C/EBPα knockout mice did not accumulate either lipid or glycogen and suffered from severe metabolic disorders that led to death by hypoglycemia[7].

In view of the coordinated inhibition of lipid and carbohydrate synthesis by TCDD and some similarities between acute symptoms of TCDD toxicity and acute response to inflammatory agents, we postulated that some of the effects of TCDD could be mediated by an alteration in the activities of C/EBP proteins, particularly in tissues engaged in energy homeostasis. We have previously observed that TCDD suppressed the levels and activity of C/EBPα while stabilizing C/EBPβ in 3T3-L1 cells during adipocyte differentiation, and this suppression of C/EBPα directly correlated with inhibition of triglyceride accumulation and fat cell differentiation[8]. In this report, we demonstrated that exposure of male C57BL mice to TCDD results in a rapid decrease in C/EBPα mRNA levels in adipose tissue and liver and in reduced DNA binding by C/EBPα.

Methods and Materials

Male C57BL/6N mice age 6-8 weeks from Simonsen Laboratories (Gilroy, CA) were used for most of the experiments. In a limited study, we used male C57BL/6-Src^{tm1sor} -/-, -/+ and their littermates, +/+ mice. Their original stock was obtained from Jackon Laboratory (Bar Harbor, ME) and from that we produced these strains through a series of backcrossings to equalize their genetic backgrounds. The strains tested are designated as N_6 strains, meaning that they have been backcrossed to C57 mice for six generations.

The method of TCDD treatment (115 μg/kg, i.p. single injection and maintained for 10 days, unless stated otherwise) has been described previously[9]. The methods for RNA isolation, northern and western blotting, gel-shift assay have also been described[8,10].

Results and Discussion

Suppression of C/EBPα and induction of C/EBPβ mRNAs by TCDD in vivo

We examined the steady-state levels of C/EBPα and C/EBPβ mRNA by northern blotting analysis in the adipose and liver of C57BL mice after a single dose (10μg/kg) of TCDD or vehicle alone. Within 24 h of TCDD treatment, C/EBPα mRNA was decreased in adipose tissue, and levels remained lower than control animals through 7 days (Fig. 1). The suppressive effect of TCDD on levels of C/EBPα mRNA was dose-dependent with an approximate ED_{50} of 3μg/kg. In contrast, TCDD treatment resulted in a 2-3 fold induction of C/EBPβ mRNA over the same period, and induction was observed at all doses tested. Neither C/EBPα nor C/EBPβ was affected at 6 h (data not shown).

A similar pattern of effect on C/EBPα and C/EBPβ mRNAs by TCDD treatment was observed in liver samples isolated from the same animals (Fig. 2). Steady-state levels of C/EBPα mRNA were decreased by TCDD, although there was some variability in the level of hepatic C/EBPα even among control animals. A larger induction of C/EBPβ mRNA (up to 8-fold higher than control) was observed in the liver than in adipose tissue and occurred within 6 h. The time course of C/EBPβ stimulation was similar to the induction of Cyp1A1 (data not shown). Changes in both C/EBPα and C/EBPβ mRNAs were dose dependent (Fig. 2).

To investigate whether such an effect of TCDD to downregulate C/EBPα is mediated by c-src kinase we treated C57-c-src deficient mice and their wild type counterpart, +/+ mice, with the same dose of TCDD and after 10 days we examined the level of C/EBP proteins in nucleus liver samples using first gel shift assay and second western blotting. The former approach yielded the data which indicated that TCDD caused downregulation of nuclear protein binding to ^{32}P-labeled C/EBP consensus response sequence in the case of N_6 src +/+ mice, but not in N_6 src-/+ mcie (Fig. 3). The results of western blotting tests on nuclear proteins with specific antibodies to C/EBPα and β showed that the level of TCDD-induced downregulation of C/EBPα was more pronounced in N_6 +/+ mice than that found in N_6-/+ mice (Fig. 4). At the same time, the level of one of C/EBPβ proteins, LAP was also downregulated. However, the percent suppression of this protein was less in N_6 +/+ than that in N_6 -/+ mice at this time point.

C/EBPα has been known to be a critical factor in cell differentiation in several well differentiated tissues such as liver, adipose tissue and pancreas[2]. Cells in these tissues are known to maintain very high titers of C/EBPα. Recently the importance of C/EBPα in keeping the normal energy homeostasis *in vivo* has been clearly demonstrated by the observation that neonates of C/EBPα knockout mice exhibit severe hypoglycemia, no glycogen storage in liver, very low PEPCK and glycogen synthase activities in liver and low fat accumulation in adipose tissue (both white and brown fat) among other abnormalities and die approximately on day 19 from the time of their birth despite the fact that they were normal when they were born[11]. It is well known that C/EBPα is one of the major nuclear transcription factors which positively controls the expression of the PEPCK gene.

Previously it was shown by this laboratory *in vitro* with the 3T3-L1 adipocyte differentiation model that the cause of the action of TCDD to downregulate C/EBPα and thereby to prevent the conversion of 3T3-L1 preadipocytes to fully differentiated adipocytes is related to its action to activate the mitogenic signaling particularly those mediated by the key transcription factors such as AP-1 and c-Myc proteins. Such a connection between upregulation of mitogenic signaling and downregulation of C/EBPα has been pointed out by others[12]. We have also shown that TCDD causes drastic downregulation of C/EBPα in mouse liver *in vivo*[8]. Thus the overall assumption that the reduced activity of c-src in-/+ mice should make those less responsive to the stimulus of TCDD to increase the mitogenic signaling and decrease C/EBPα agrees well with the observed tendency of these c-src deficient mice.

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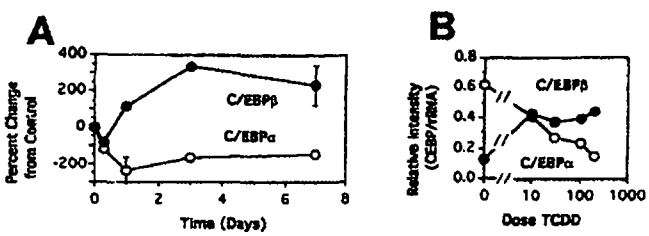


FIG. 1. Northern blot analysis of the effect of TCDD on C/EBPα and C/EBPβ mRNA in adipose tissue of male C57BL/6 mice. (A) Time course of the effect of 10μg/kg TCDD. For this purpose the blots were analyzed through densitometry and normalized to the corresponding rRNA readings. (B) Effect of TCDD dose on expression of C/EBPα and β after 3 days.

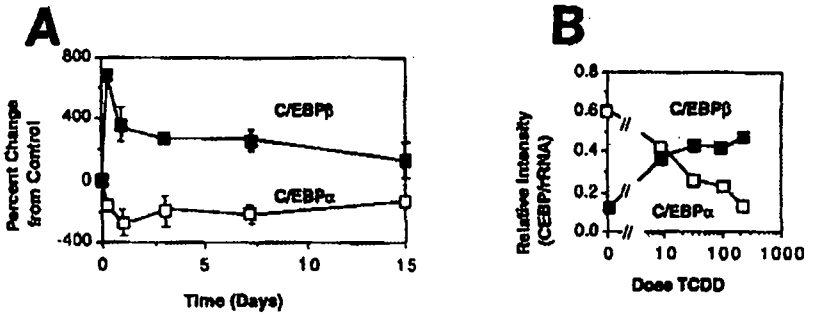
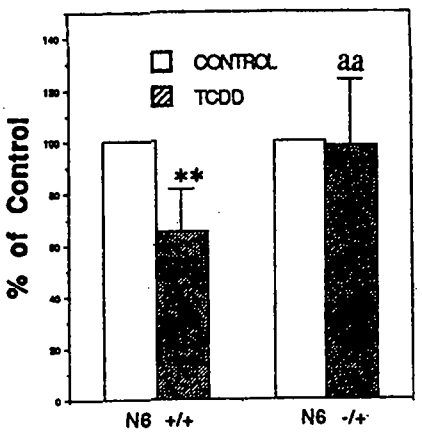


FIG. 2. Northern blot analysis of the effect of TCDD on C/EBPα and β mRNA in liver. See Figure 1 caption for additional explanations.

FIG. 3. (Left figure below.) Effect of 115 μg/kg TCDD single treatment on the levels of C/EBP protein binding to C/EBP recognition site on ^{32}P-labeled oligonucleotide probe at day 10 as assessed via gel-shift assay. For this experiment liver samples from individual mice from c-src deficient (N6 -/+) and their wild littermates (N6 +/+) C57BL/6 strains were analyzed and the results are shown as densitometric band intensities normalized to control. Values shown are mean of two independent experiments. ** indicate that TCDD caused a significant change as compared to control at $p \leq 0.05$. "aa" signify that the value is different from the corresponding figure from N_6 +/+ treated mice.



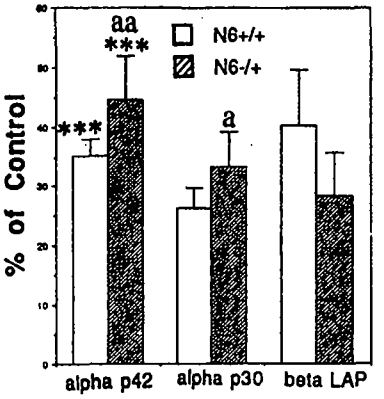


FIG. 4. (Right figure above.) Western blot assessment of the relative levels of C/EBPα and β proteins present in the hepatic nuclear fractions obtained from c-src deficient mice (N6/+) and their wild littermates (+/+). *** indicate that the effect of TCDD is significant at $p<0.01$ as compared to control. "a" or "aa" indicate that the values for N_6/+ were different from the corresponding ones from N_6 +/+ TCDD-treated mice.