Biochemical Pharmacology, Vol. 31, No. 15, pp. 2459–2462, 1982.
Printed in Great Britain.

0006-2952/82/152459-04 $03.00/0

DIFFERENTIAL EFFECT OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN ON NUCLEAR RNA POLYMERASE ACTIVITY IN THE RAT LIVER AND THYMUS

RABINDER N. KURL, JOHAN LUND, LORENZ POELLINGER and JAN-ÅKE GUSTAFSSON
Department of Medical Nutrition, Karolinska Institute, P.O. Box 60400, S-104 01 Stockholm, Sweden

(*Received* 20 *May* 1981; *accepted* 30 *January* 1982)

Abstract—An increase in RNA polymerase B activity in hepatic cell nuclei was observed after a single intravenous injection of 1 μg/rat of TCDD. The enzyme activity was about 70% above the control value at 1 hr after injection and then declined rapidly. A secondary increase was evident at 24 hr. Prior to the secondary increase in RNA polymerase B activity, there was an increase in RNA polymerase A activity which was about 125% above the control value. The initial increase in RNA polymerase B activity was sensitive to both α-amanitin and actinomycin D injected 30 min before TCDD administration. In the thymus, an increase in RNA polymerase B activity was observed 4 hr after injection (25% above control value) but thereafter it declined and at 24 hr it was about 30% below the control value. RNA polymerase A activity was inhibited as early as 1 hr after injection but had returned to the control value at 4 hr and then paralleled RNA polymerase B activity. Thus, TCDD stimulated RNA synthesis in the rat liver but the drug inhibited RNA synthesis in the rat thymus. Since these effects were detected as early as 1 hr after TCDD administration it is proposed that TCDD action requires transcriptional response and that this response may represent a primary site of TCDD action in the cell. The cellular entities or mechanisms which TCDD uses to alter transcription in the cell nucleus remain to be determined.

A number of studies indicate that polycyclic aromatic hydrocarbons (PAH) like TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) manifest their effects by binding to cytosolic proteins which in turn are translocated to the nucleus [1–4]. The levels of these cytosolic binding proteins correlate well with the induction of aryl hydrocarbon hydroxylase (AHH) activity in the mouse liver [1]. On the contrary, very minimal levels of AHH are induced in the rodent thymus by TCDD [5] though cytosolic proteins binding TCDD are present in the thymus [6].

The stimulating effect of PAH on the RNA content of the liver [7] and protein synthesis by the liver [8] have also been described as has evidence that these agents augment synthesis of several liver enzymes [9–14]. These data suggest that the locus of this PAH effect is the RNA synthesizing machinery [15, 16]. Since the TCDD binding proteins seem to behave in a manner analogous to that reported for steroid binding proteins [17–19], it is possible that the genome is affected when the TCDD binding proteins are translocated to the nucleus.

Our interests have also centred on the observation that an injected PAH, e.g TCDD, may affect similar processes in two different organs in the same animal in opposite directions. Administration of TCDD appears to augment synthesis of specific liver constituents while simultaneously causing marked involution of the thymus. Experiments were therefore initiated to ascertain whether these gross changes in tissue composition and size were reflected in demonstrable alterations at a subcellular level and to assess the enzymatic basis of such changes. At the same time, to assess the functional integrity of the TCDD binding protein, it seemed logical to investigate the effect of TCDD on early transcriptional events. In this paper we present data on the effect of TCDD on nuclear endogenous activity of RNA polymerases A and B in the rat liver and thymus.

MATERIALS AND METHODS

Chemicals: [5-^{3}H]UTP (sp.act. 2 Ci/mmole) was purchased from the Radiochemical Centre (Amersham, U.K.). ATP, CTP, GTP, α-amanitin and actinomycin D were obtained from Boehringer (Mannheim, F.R.G). TCDD was a gift from Dr. Sören Jensen (Wallenberg Laboratory, University of Stockholm, Sweden). All other reagents used were of analytical grade.

Animals: Three-week-old male Sprague–Dawley rats weighing 50–60 g were used.

Procedures: Rats were injected intravenously with a single dose of 1 μg/rat of TCDD in a total volume of 100 μl of isotonic saline containing 1% (v/v) Triton X-100. Control rats were injected with the vehicle only. At various times post-injection the rats were killed by cervical dislocation and the livers and thymus glands removed on to ice. Hepatic cell nuclei were prepared by the method of Blobel and Potter [20]. Briefly, the liver tissue was finely minced and homogenised in Buffer A (0.5 M Tris, 0.025 M KCl, 0.005 M $MgCl_2$, 0.25 M sucrose, pH 7.5, 2 ml/g of tissue) in a Potter–Elvehjem glass homogeniser fitted

with a Teflon pestle. The homogenate was filtered through four layers of cheese cloth and well mixed with 2 vol. of Buffer B (0.05 M Tris, 0.025 M KCl, 0.005 M $MgCl_2$, 2.3 M sucrose, pH 7.5) to give a final concentration of 1.62 M sucrose. This was underlaid with one volume of Buffer B and centrifuged for 40 min at 105,000 *g* in a Beckman L3-50 ultracentrifuge. The supernatant was discarded and the pellet was suspended in TEDG buffer (0.05 M Tris, 0.001 M EDTA, 0.005 M dithiothreitol and 25% (v/v) glycerol, pH 7.9).

Thymic nuclei were prepared by a hypotonic shock as described previously [21]. Endogenous RNA polymerase A and B activities were measured in the liver and thymic nuclei as reported earlier [22–24]. The two enzyme activities were distinguished by the activation of RNA polymerase B by Mn^{2+} and high ionic strength and its selective inhibition by low concentrations of α-amanitin (1.25 μg/ml).

RNA polymerase activity in hepatic cell nuclei was measured by the method of Kurl and Borthwick [22] enabling the separate quantification of RNA polymerase A and B in the presence of each other. The reaction mixture contained in a volume of 30 μl, 0.075 μmole each of ATP, CTP and GTP, 0.005 μmole of [^{3}H]UTP (0.5 μCi), 1.25 μmole of Tris–HCl buffer, pH 7.9, containing 0.5 μmole of 2-mercaptoethanol and 5% (v/v) glycerol. For the measurement of RNA polymerase A activity, the same volume of the reaction mixture also contained 0.32 μmole of $MgCl_2$, 3 μmole of KCl and 0.1 μg of α-amanitin. Assays carried out under high salt conditions (to measure RNA polymerase B activity) contained 0.24 μmole of $MnCl_2$ and 16 μmole of $(NH_4)_2SO_4$ in the same volume of reaction mixture.

Nuclei were added in a volume of 50 μl to the reaction mixture at 0° and incubated at 37° for 15 min. The reaction was stopped by immersing the incubated samples in crushed ice. Aliquots (50 μl) of the incubation mixture were spotted on 2.5 cm discs of Whatman No. 1 filter paper and immersed for 30 min with occasional stirring in 10% (w/v) trichloroacetic acid containing 1% (w/v) tetrasodium pyrophosphate. The discs were washed twice with 5% (w/v) trichloroacetic acid containing 1% (w/v) tetrasodium pyrophosphate, once with alcohol and finally with ether. After drying the filter papers in a stream of air, each was incubated for 20 min at 60° in a polypropylene scintillation vial containing 0.5 ml of 1 M Hyamine 10-X to solubilize the radioactivity. The latter was measured in a liquid scintillation counter.

RNA polymerase activity in isolated thymic cell nuclei was measured as described above except that RNA polymerase A activity was measured in the presence of 0.24 μmole $MgCl_2$ and 4 μmole of KCl whereas RNA polymerase B activity was assayed in the presence of 0.32 μmole $MnCl_2$ and 16 μmole $(NH_4)_2SO_4$.

DNA determinations were carried out by the method of Burton [25].

RESULTS

The activity of RNA polymerase was measured in order to determine whether the presence of TCDD in the nucleus influenced transcription. In the hepatic nuclei TCDD did not affect RNA polymerase A activity during the first hour of treatment. Stimulation of RNA polymerase B activity reached maximal levels at 1 hr after injection which was about 70% above control. A secondary increase in polymerase B activity was evident at 24 hr also. Prior to the secondary increase in RNA polymerase B activity there was an increase in RNA polymerase A activity which was about 125% above the control activity (Fig. 1).

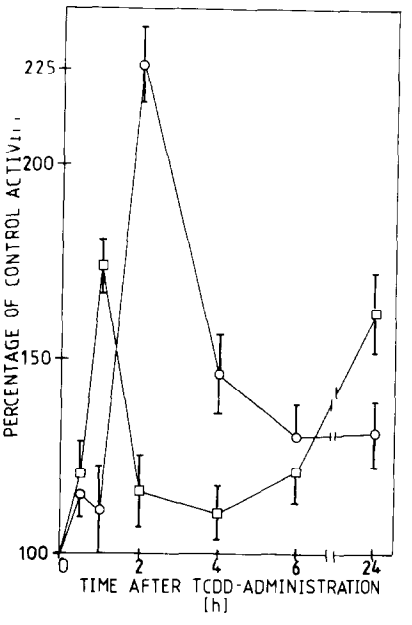

Fig. 1. Effect of TCDD on the activities of RNA polymerase A (○——○) and RNA polymerase B (□——□) in the rat liver. Rats were injected intravenously with either 1 μg/rat of TCDD or vehicle only and killed at varying times. Hepatic nuclei were isolated and assayed for endogenous enzyme activity as described in Materials and Methods. Control activity (100%-vehicle treated animals) in terms of [^{3}H]UMP incorporation were 72139 ± 4454 dpm/mg DNA for RNA polymerase A and 365361 ± 9540 dpm/mg DNA for RNA polymerase B. Values are mean ± S.E.M. (n = 6).

In the thymic nuclei RNA polymerase B activity fluctuated around control values for the first 2 hr. An increase of about 25% was evident at 4 hr; thereafter the enzyme activity declined and at 24 hr it was 30% below the control value. RNA polymerase A activity was inhibited at 1 hr by about 40% and this inhibition was maximal at 2 hr (about 55% below control value); at 4 hr the enzyme activity had returned to the control value. A secondary inhibition in the activity was observed at 24 hr (Fig. 2).

DISCUSSION

The premise of the present investigation is to decipher the primary site of action of TCDD in the liver and the thymus where the effects of the drug are different.

We have shown that TCDD binds to nuclear proteins both in the hepatocytes and thymocytes [3, 6].

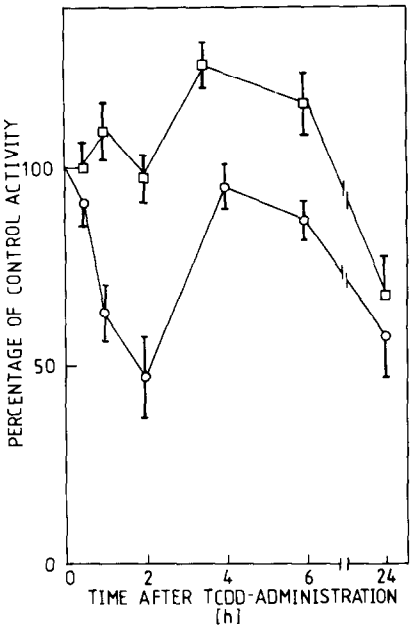

Fig. 2. Effect of TCDD on the activities of RNA polymerase A (○——○) and RNA polymerase B (□——□) in the rat thymus. Rats were injected intravenously with either 1 μg/rat of TCDD or vehicle only and killed at varying times. Thymic nuclei were isolated and assayed for endogenous enzyme activity as described in Materials and Methods. Control activity (100%-vehicle treated animals) in terms of [^{3}H]UMP incorporation were 3009 ± 302 dpm/mg DNA for RNA polymerase A and 39507 ± 3431 dpm/mg DNA for RNA polymerase B. Values are mean ± S.E.M. (n = 6).

Nuclear proteins have been implicated in the modulation of species and tissue specific transcription in both normal [26] and malignant cells [27]. Thus, it seemed logical to investigate the effect of TCDD on early transcriptional events. The enhancement of RNA polymerase B activity in isolated hepatic nuclei by about 70%, 1 hr after TCDD treatment, is the first direct demonstration of the involvement of RNA synthesis in the primary action of this drug. It is probable that the increase in enzyme activity observed in isolated nuclei is a consequence of changes in the liver chromatin, arising directly from the interaction of TCDD and its protein with specific sites in the chromatin.

These results are compatible with those reported for 3-methylcholanthrene (3MC), a congener of TCDD [28], in the rat liver. These effects could be due to either an increase in template efficacy [29] or a direct effect on the enzymes [30] as observed with 3MC. The transient TCDD-induced increase in RNA polymerase B activity in rat liver cell nuclei represents one of the earliest responses of this tissue to PAH. The time at which the effect was observed suggests that it may represent the synthesis of hnRNA destined to produce the species of mRNA involved in the generation of the effects leading to synthesis of the cytochrome P-450-dependent microsomal enzymes. The reason for the biphasic response of the RNA polymerase B activity to TCDD treatment *in vivo* in the liver is at present obscure. One can speculate that the first peak of activity (Fig. 1) at 1 hr produces RNA, which in turn induces the later responses of the tissue to the drug.

The ability of a cell nucleus to synthesize RNA can be limited by various mechanisms such as (1) substrate (nucleotide) concentration, (2) amount of available DNA to serve as a template, (3) amount of active enzyme, and (4) the efficiency of the available enzyme in polymerizing nucleotides. By using an excess of nucleotides in an RNA polymerase assay *in vitro* the first possibility should be eliminated.

The effect of TCDD on the thymus is neither mediated through the pituitary nor the adrenals, since atrophy of the thymus occurred in TCDD-exposed rats that were hypophysectomised or adrenalectomised [31]. In addition, the effect of TCDD on the thymus is not caused by reduced food intake, production of thymic hormone or via induction of α-fetoprotein [32]. Glucocorticoids like dexamethasone do not compete for the TCDD binding protein [3] and vice versa [33].

How TCDD induces thymic atrophy is still an enigma, but the present results may suggest the involvement of RNA synthesis. Thymic atrophy is also induced by glucocorticoids and they may be mediating their effects by inhibiting glucose [34] and α-aminoisobutyric acid [35] transport and oxidative ATP production [36]. All these processes share in common a dependence on protein and RNA synthesis [37]. It is possible that the TCDD-binding protein complex initiates or increases the synthesis of regulatory proteins which directly mediate the observed metabolic effects.

As there is a paucity of data on the number and function of the genes regulated by the TCDD binding protein it is difficult to explain the differences in transcriptional activity observed in the rat liver and thymus. It is possible that the binding of TCDD to the cytosolic protein and translocation to the nucleus with either sustained expression or repression of one or more genes controlled by the binding protein leads to the toxic responses characteristic of TCDD.

In the liver maximum cell nuclear uptake of TCDD is observed at 2 hr after injection [3] whereas the maximal effect of TCDD on RNA polymerase B activity reported in the present study occurred at 1 hr. This discrepancy in results may be explained by the fact that we injected about a thousand fold higher dose of TCDD for the measurement of RNA polymerase activity when compared to the dose used for studies on uptake of TCDD. In the rat thymus maximal uptake of TCDD was observed at 3 hr after injection [6]. In this organ the effect on RNA polymerase A activity was observed prior to that on RNA polymerase B activity. In the liver, on the contrary, RNA polymerase B activity was affected first. Perhaps the RNA polymerase B activity leads to the synthesis of hnRNA which in turn produces mRNA which codes for a protein leading to an increase in RNA polymerase A activity as is known for steroid hormones [23]. In the thymus, TCDD could be acting directly on RNA polymerase A. The delayed effect on RNA polymerase B activity observed in the thymus 4 hr after injection (which is 3 times less as compared to the increase in RNA polymerase B activity at 1 hr in the liver—Fig. 1)

may lead to RNA synthesis including mRNA which is translated into enzymes such as AHH activity and also another factor which may be responsible for switching off RNA synthesis at 6 hr after the injection of TCDD.

In the rat liver, the PAH-induced synthesis of enzymes can be obliterated with actinomycin D treatment [38]. The induction of a battery of enzymes in response to TCDD in the rodent liver may be due to the stimulation of RNA synthesis whereas inhibition of RNA synthesis as observed in the thymus may be the controlling factor for the minimal increase in enzyme activity in the thymus.

Acknowledgements—R.N.K. is grateful to Ekhagastiftelsen and the Swedish Institute for fellowships. This work was supported by a grant from the Swedish Cancer Society.

REFERENCES

1. A. P. Poland, E. Glover and A. S. Kende, *J. biol. Chem.* **251**, 4936 (1976).
2. W. F. Greenlee and A. Poland, *J. biol. Chem.* **254**, 9814 (1979).
3. R. N. Kurl, L. Poellinger, J. Lund and J.-Å. Gustafsson, in *Biochemistry, Biophysics and Regulation of Cytochrome P-450* (Eds. J. Å. Gustafsson *et al.*), p. 151. Elsevier, Amsterdam (1980).
4. B. Tierney, D. Weaver, N. H. Heintz, W. I. Schaeffer and E. Bresnick, *Archs Biochem. Biophys.* **200**, 513 (1980)
5. A. Poland and E. Glover, *Molec. Pharmac.* **17**, 86 (1980).
6. R. N. Kurl, L. Poellinger, J. Lund and J.-Å. Gustafsson, in *Chlorinated Dioxins and Related Compounds. Impact on the Environment* (Eds. O. Hutzinger, E. Merian and R. W. Frei), pp. 437–447. Pergamon Press, Oxford (1982).
7. E. Bresnick, M. E. Synerholm and G. T. Tizard, *Molec. Pharmac.* **4**, 218 (1968).
8. J. R. Fouts and L. A. Rogers, *J. Pharmac. exp. Ther.* **131**, 282 (1965).
9. A. P. Alvares, G. R. Schilling, W. Levin and R. Kuntzman, *Biochem. biophys. Res. Commun.* **29**, 521 (1967).
10. S. Orrenius, M. Das and Y. Gnosspelius, in *Microsomes and Drug Oxidations* (Eds. J. R. Gillette *et al.*), p. 251. Academic Press, New York (1969).
11. J. E. Giclen and D. W. Nebert, *J. biol. Chem.* **246**, 5189 (1971).
12. H. S. Marver, in *Microsomes and Drug Oxidations* (Eds. J. R. Gillette *et al.*), p. 495. Academic Press, New York (1969).
13. D. W. Nebert and J. E. Gielen, *J. biol. Chem.* **246**, 5199 (1971).
14. S. T. Jacob, M. B. Scharf and E. S. Vessel, *Proc. natn. Acad. Sci. U.S.A.* **71**, 704 (1974).
15. E. Bresnick, *Molec. Pharmac.* **2**, 406 (1966).
16. H. V. Gelboin, J. S. Wortham and R. G. Wilson, *Nature, Lond.* **214**, 281 (1967).
17. J. Gorski and F. Gannon, *A. Rev. Physiol.* **38**, 425 (1976).
18. K. R. Yamamoto and B. M. Alberts, *A. Rev. Biochem.* **45**, 721 (1976).
19. W. V. Vedeckis, W. T. Schrader and B. W. O'Malley, *Biochem. Acts Horm.* **5**, 321 (1978).
20. G. Blobel and V. R. Potter, *Science* **154**, 1662 (1966).
21. C. R. Wira and A. Munck, *J. biol. Chem.* **249**, 5328 (1974).
22. J. R. Tata and B. Baker, *Expl. Cell Res.* **83**, 125 (1974).
23. N. M. Borthwick and P. A. Bell, *FEBS Lett.* **60**, 396 (1975).
24. R. N. Kurl and N. M. Borthwick, *J. Endocr.* **85**, 519 (1980).
25. K. Burton, *Biochem. J.* **62**, 315 (1956).
26. J. Paul and R. S. Gilmour, *J. molec. Biol.* **34**, 305 (1968).
27. N. C. Kostraba and T. Y. Wang, *Cancer Res.* **2**, 2348 (1972).
28. R. G. Wilson, S. I. Wortham and H. V. Gelboin, *Adv. Enzyme Reg.* **5**, 385 (1967).
29. J. C. Madix and E. Bresnick, *Biochem. biophys. Res. Commun.* **28**, 445 (1967).
30. P. A. Liberator and E. Bresnick, *Chem.-Biol. Interact.* **35**, 93 (1981).
31. M. J. van Logten, B. N. Gupta, E. E. McConnell and J. A. Moore, *Toxicology* **15**, 135 (1980).
32. J. G. Vos, *CRC Crit. Rev. Tox.* **5**, 67 (1977).
33. R. A. Neal, P. W. Beatty and T. A. Gasiewicz, *Ann. N. Y. Acad. Sci.* **320**, 204 (1979).
34. A. Munck, *J. biol. Chem.* **243**, 1039 (1968).
35. D. A. Young, S. Giddings, A. Swonger, G. Klurfeld and M. Miller, *Excerpta Med. Int. Congr. Ser.* **210**, 843 (1971).
36. S. K. Nordeen and D. A. Young, *J. biol. Chem.* **251**, 7295 (1976).
37. A. Munck and A. Leung, in *Receptors and Mechanism of Action of Steroid Hormones.* II (Ed. J. R. Pasqualini), p. 311. Marcel Dekker, New York (1977).
38. H. V. Gelboin and N. R. Blackburn, *Cancer Res.* **24**, 356 (1964).