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INFLUENCE OF 2,3,7,8-TCDD[1] ON THE PROTEIN COMPOSITION OF THE PLASMA MEMBRANE OF HEPATIC CELLS FROM THE RAT

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SUMMARY: To understand the mechanism of toxic action of TCDD, a serious environmental pollutant, rats were injected with 25 μg/kg and their livers removed 10 days post-treatment. A canaliculi-rich, plasma membrane fraction was prepared and its protein composition was examined using SDS-polyacrylamide gel elctrophoresis. As a result of close comparison between untreated and treated preparations it was concluded that many protein levels were reduced in the membrane from the TCDD treated rats. This phenomenon was confirmed by the finding that there is an overall reduction in binding of ^{3}H-concanavalin A to the TCDD-treated plasma membrane.

TCDD[1] has been considered to be one of the most serious environmental pollutants (1). Its LD_{50} to guinea pigs has been established to be 0.6 μg/kg, making it the most toxic small size, man made chemical known to exist (2). Furthermore it is known to be teratogenic, acnegenic and carcinogenic (3). Its toxic action is unusual in two respects. First, after a single administration of TCDD, the animal does not show any overt external poisoning symptoms until its death which occurs usually 10 to 30 days post-treatment. Second, the treated animal begins to lose its body weight after a few days. Numerous research attempts have been made to understand the basis of its toxic action in recent years. The most conspicuous feature of TCDD action is the induction of aryl hydrocarbon hydroxylase (AHH) activity in several tissues including the liver *in vivo* (4,5). The above change is accompanied by massive increases in microsomal protein including cytochrome P_{448} (5) and UDP-glucuronosyltransferase (6). It was originally found by Poland and Glover (7) that there is a cytosolic receptor which specifically binds with TCDD and this receptor binding apparently leads to other biochemical changes that occur as a result of TCDD poisoning *in vivo*. This subject area has been extensively reviewed (8,9). On the other

1. Abbreviation of 2,3,7,8-tetrachlorodibenzo-p-dioxin

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hand, there is an indication that the above induction of microsomal proteins and functions do not adequately explain why TCDD is toxic. For instance, systems of the most sensitive species, guinea pig are not known to be highly susceptible to induction, while those of more resistant species such as hamster are (10). Indeed there are many inducers which do not show high toxicities or cause body weight losses. In view of the importance of TCDD to environmental toxicology and the lack of a clear cut explanation for its toxic action we have examined another potential site of TCDD action, plasma membranes, in this work.

MATERIALS AND METHODS

Male Sprague-Dawley rats (125-150 g) were obtained from Spartan Research Animals Inc. Haslett, Michigan. Food (Wayne Lab Blocks, Chicago, Ill.) and water were provided ad libidum and the animals were maintained on a 12 hour light, 12 hour dark cycle.

Animals were dosed intraperitoneally with 25 μg/kg TCDD (Dow Chemical Co. Midland, MI), dissolved in a 1:9 solution of acetone: corn oil; controls were dosed with an equal volume of vehicle. At 10 days post-treatment the animals were sacrificed by decapitation and the hepatocyte plasma membranes (PM) isolated according to the procedure of Yunghans and Morré (11). Examination by electron microscopy and marker enzyme assays (Na-K ATPase and ^{3}H-concanavalin A binding) verified the presence of PM vesicles and absence of significant mitochondial and/or microsomal contamination.

Gel electrophoresis was performed with a Bio-Rad 221 Dual Slab Gel system using the method of Laemmli (12) as modified by Hoefer Scientific (13). Gels were subsequently dried using a Bio-Rad Gel Slab Dryer (Model 224) for densitometric scanning. The bands resulting from Coomassie Blue staining were scanned for their relative intensity, utilizing an ACD-18 Gelman Densitometer and the areas under the peaks integrated for comparison purposes between plasma membrane preparations.

Concanavalin A binding was determined by mixing 25 μg membrane (in 0.25 M sucrose) with a 0.21 μg ^{3}H-concanavalin A (New England Nuclear, specific activity 25 Ci/mmole) to a total reaction volume of 0.2 ml. Each reaction tube was subsequently incubated for 10 min. at 37°C after which the reaction was terminated with the addition of 8 ml cold 0.1 M Tris-HCl. The mixture was then quickly passed through 0.45 μ HA filter (Millipore Corp., Bedofrd, MA), washed with an additional aliquot of 8 ml Tris-HCl, allowed to air dry, and the remaining radioactivity assayed. Specific binding was determined in the presence of .01 M alpha methyl mannoside (Sigma Chemical Co., St. Louis, MO). Parallel tubes were incubated for 5 to 10 min. in the presence and absence of α-methyl mannoside (αmm) before the addition of ^{3}H-concanavalin A. Specific binding was calculated by subtracting the radioactivity remaining in the tube without α-mm. Specific binding was on the order of 3-6% of the total radioactivity added.

RESULTS AND DISCUSSION

The basic pattern of electrophoresis of the SDS-treated hepatic plasma membrane is shown in Figure 1. The intensities of bands were measured by using a densitometric technique and the result is shown in Table 1. The most striking change observed here was that the levels of many protein bands in the TCDD treated electro-

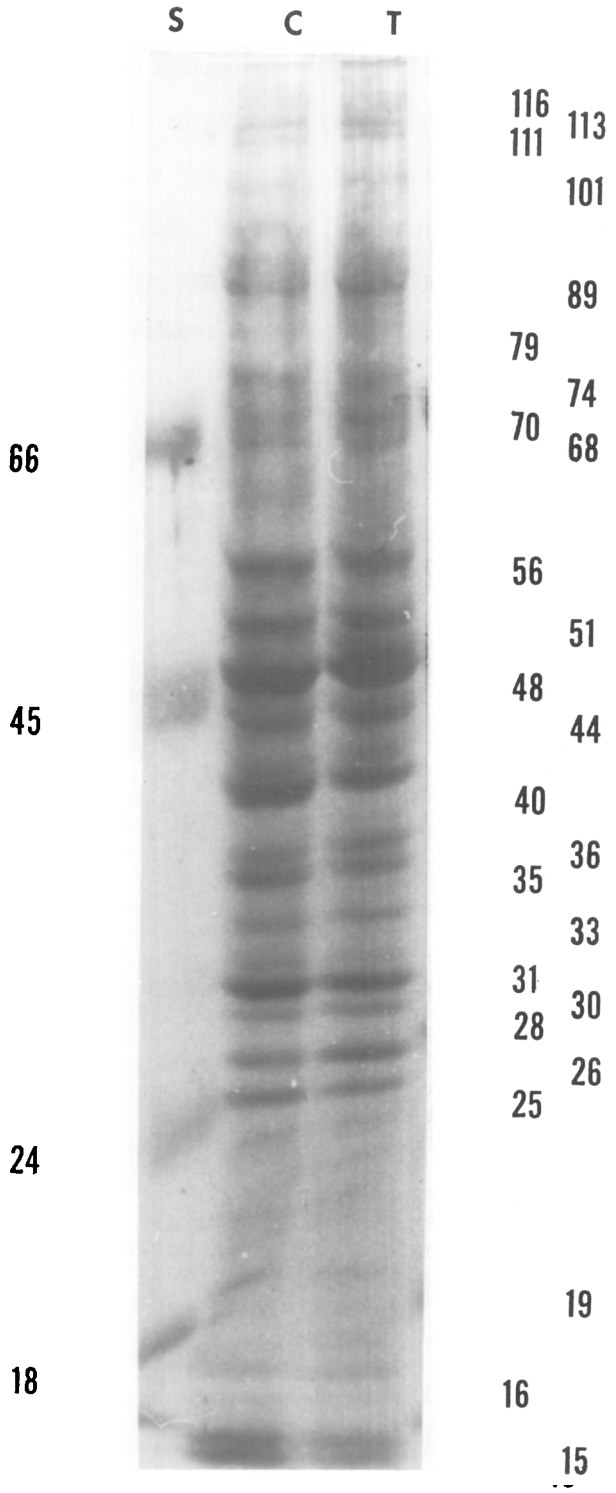

Fig. 1. SDS-polyacrylamide slab gel electrophoresis patterns of hepatic plasma membranes from TCDD-treated (T), control (C) rats. Standard protein mixture (S) contained bovine serum albumin (66k), egg albumin (45k), trypsinogen (24k) and β-lactoglobulin (18.4k).

Table 1. Effect of *in vivo* treatment of TCDD on the protein composition of hepatic plasma membrane. The SDS gel electropherograms such as the ones shown in Fig. 1 were scanned by a densitometer and the peak area corresponding to each band was integrated and is expressed as percentages of the total protein found in this molecular weight range.

Molecular Weight (X1000)	Control	TCDD-treated	% Increase (+) or decrease (-) against control
116	0.85	3.91	+360%
113	1.17	6.45	+428%
111-108	6.08	3.36	- 45%
104-101	2.96	0.99	- 67%
93-87	8.01	8.98	+ 12%
79	0.82	1.71	+108%
74	1.84	1.87	+ 2%
70	1.54	2.28	+ 48%
68	0.95	1.09	+ 15%
67-57	0.86	0.88	+ 2%
56	3.95	3.09	- 22%
51	3.60	3.14	- 13%
48	9.34	8.19	- 12%
45	3.66	2.12	- 42%
40	5.38	2.58	- 52%
36	2.83	0.61	- 78%
35	6.88	4.48	- 35%
33	4.76	2.66	- 44%
30-31	10.79	10.05	- 7%
28	2.31	2.62	+ 13%
26	3.23	7.75	+140%
25	3.42	3.28	- 4%
16-24	7.16	10.35	+ 45%
15	5.86	4.68	- 20%
14[a]	1.77	2.59	+ 46%

[a]Bond 14k not pictured in Fig. 1.

pherogram are severely depressed while some other bands are intensified. Two features are pertinent to mention. First the band 48k has been found to be consistent among all plasma membranes studied (14), and therefore may be used as an internal standard. Second, the absence of an increase of band 54 to 56k in the treated membrane which corresponds to cytochrome P_{448}, indicates minimal contamination of the plasma membranes with microsomes. This electrophoresis experiment was repeated at least 6 times each using a different preparation of rat livers and the above ten dency was confirmed.

To ascertain that such changes are occurring truly on the plasma membrane a quantitative assay on ^{3}H-concanavalin A binding with the surface glycoproteins (15) was conducted. In the result summarized in Fig. 2, one can see that the overall rate of specific ^{3}H-concanavalin A binding to the TCDD treated plasma membrane was depressed as compared to the control. The levels of ^{3}H-concanavalin A binding at

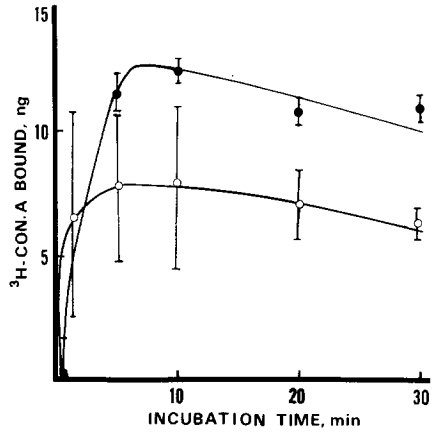

Fig. 2. Time course of ^{3}H-concanavalin A binding to liver plasma membrane from normal (●) and TCDD-treated (o) rats. Only specific binding was measured in this experiment. Results of 4 independent experiments. The vertical lines show the range of standard error for each point.

10 minutes were measured by using different batches of rat preparations. The results are shown in Table 2. The TCDD-induced reduction in binding was particularly significant in the category of specific binding, but reduction in the total binding was also noticeable.

It has been previously shown by our group, in collaboration with Dr. R. Peterson (16,17), that the activities of two plasma membrane bound ATPases, Na-K and Mg-ATPase in the rat liver were reduced by *in vivo* treatment of TCDD. The above study also showed that the biliary transport of ouabain, a model neutral substrate, was reduced at the same time. Since all these events may be traced to the function of the hepa-

Table 2. Effect of *in vivo* treatment of TCDD on ^{3}H-concanavalin A binding to rat liver plasma membrane[a]

Source of Rats	ng ^{3}H-Concanavalin A bound/25 µg protein ± S.E.[b]	
	Total	Specific[c]
Control	12.0 ± 4.3	11.0 ± 3.0
TCDD-treated	9.5 ± 2.8	6.6 ± 2.9[d]

a ^{3}H-Concanavalin A binding was measured 10 min. after addition of the labeled ligand. Experimental details as given in Materials and Methods section.

b Average ± standard error of 4 separate experiments.

c Specific binding is the difference between binding in the absence and presence of α-methyl mannoside.

d Significantly different from control at $P < 0.01$.

tic plasma membrane, it may be safe to conclude that the changes in their activities are caused by TCDD's action on the plasma membrane in vivo. Whether such an action of TCDD is directly related to its toxic manifestations in various animal species is a matter of conjecture at this stage. Knowing the importance of plasma membrane surface proteins in maintaining normal cellular functions, however, it appears to be imperative that the meaning of such TCDD-induced changes be carefully examined in the future.

As for the meaning of the reduction of concanavalin A binding to the plasma membrane by TCDD treatment there appears to be a possibility to logically relate the event to hapatocarcinogenesis, i.e. one of the toxic manifestations of TCDD. That is, as shown by Pitot et al. (18) TCDD has potent action as a tumor promoter in the rat liver. The loss of the surface glycoproteins has been linked to the loss of cell-cell recognition, and thereby increase the chance for escape from contact inhibition. In fact many precancerous or transformed cells are known to have reduced surface glycoproteins (19).

The most significant aspect of our finding is that there are many other unidentified proteins in the plasma membrane that showed marked decreases or increase in quantities as shown by the result of electrophoresis experiments. Such significant changes in membrane proteins may offer additional clue to the puzzle of TCDD induced toxic actions in animals.

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