Receptor-Mediated Carcinogenesis and Dioxin

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1. Introduction

Dioxin has routinely been referred to as a paradigm for "receptor-mediated" carcinogens, especially those whose actions are mediated through the Ah-receptor. Numerous theories exist on the effect that receptor-mediated events will have on the shape of dose-response curves for carcinogenesis (see numerous articles cited in reference 1). Most of these theories have not been rigorously developed and applied to the data available on the effects of dioxin. In this paper, we develop a mathematical model for gene expression which, in our opinion, is the minimum model necessary to discuss theoretical issues relating receptor binding to dose-response. The implications of dose-related changes in this model on gene expression will be discussed.

2. A Simple Theoretical Model for Gene Expression

Figure 1 illustrates the simplest comprehensive mathematical model of receptor-mediated gene expression. It takes into account the necessary steps of binding of natural and xenobiotic ligands, protein synthesis and degradation, and metabolism of the xenobiotic ligand (2). This model deals solely with gene expression at the level of the cell and not with the complicated issues of dose delivery and metabolic activation; these must be discussed in the context of modeling the effects of dioxin directly.

Our theoretical model (see Figure 1) describes the expressio of some protein (P) which is produced through three possible pathways - direct constitutive control which is not receptor-mediated, receptor mediated control via a natural ligand (N) and receptor-mediated control via a xenobiotic ligand (X) - and removed via proteolysis. The net rate of production of the gene product, P, is given by the differential equation

$$\frac{dP}{dt} = v_{dc} + v_{RN} + v_{RX} - v_{p}$$

where vdc is the constant rate of production of protein by the mechanism that does not involve the receptor, vRN is the rate of induction of the protein by the natural ligand, vRX is

the rate of induction of the protein via the xenobiotic ligand and vp is the rate of proteolysis of the protein. The two receptor-mediated induction rates are the results of other formulae dealing with binding to the receptor and binding to DNA as follows.

If we assume equilibrium binding of the natural and xenobiotic ligands to the receptor and that there are I binding sites for either ligand on each receptor molecule, the concentration of natural ligand-receptor complex (RN) can be modeled by the equation

$$RN = \frac{R_{tot} - RX}{1 + (K_N / N)^I}$$

and the concentration of the xenobiotic ligand-receptor complex (RX) can be modeled by the equation

$$RX = \frac{R_{tot} - RN}{1 + (K_X / X)^I}$$

where KN is the dissociation constant for RN and KX is the dissociation constant for RX. These are equilibrium equations of the Hill type (3) and I is referred to as the Hill exponent.

Assuming that the receptor complexes must bind to n binding sites on DNA in order to initiate transcription of the coding region, RN and RX compete for binding to the same DNA sequence and the DNA complexes are at steady state, the rate of induction of protein is given as

$$v_{RN} = \frac{V_{max}}{1 + \left(K_{RN}/RN\right)^n \left[1 + \left(RX/K_{RX}\right)^n\right]}$$

for the natural ligand and as

$$v_{RX} = \frac{V_{max}}{1 + \left(K_{RX}/RX\right)^n \left[1 + \left(RN/K_{RN}\right)^n\right]}$$

for the xenobiotic ligand where Vmax is the maximum rate of induction of the protein, KRN is the apparent dissociation constant for the natural ligand-receptor complex with DNA and KRX is the apparent dissociation constant for the xenobiotic ligand-receptor complex with DNA. It is assumed that constitutive expression via the non-receptor pathway is independent of this expression (it does not affect Vmax).

We assume that degradation of the protein is accomplished by the action of proteolytic enzymes with p binding sites for protein P. Again using Hill equations, the corresponding rate of proteolysis can be expressed as

$$v_p = \frac{E_{max}}{1+(K_p/P)^p}$$

where Emax is maximum rate of proteolysis and Kp is the protein concentration which produces half of the maximum reaction rate.

Similarly, metabolism of the xenobiotic ligand can be modeled using a Hill equation. The rate of loss of the ligand X following a bolus dose is given

$$\frac{dX}{dt} = -\frac{M_{max}}{1+(K_m/X)^m}$$

where Mmax is the maximum rate of metabolism, Km is the concentration of xenobiotic ligand at which half of the maximum rate is achieved and m is the corresponding Hill exponent.

3. Dose-Response Profiles

Values of the model's parameters were varied in numerous ways to reflect alternative mechanisms of expression of the protein. Changing the assumptions had dramatic effects on the computed response to a bolus dose of the xenobiotic ligand. Even in cases where all processes in the model exhibit hyperbolic kinetics (all of the Hill exponents equal to 1), the dose-response curves can appear sigmoidal but actually be linear with a positive slope at low doses. The slope of the curve only approached zero at low dose, indicative of a threshold for response, if binding of the xenobiotic ligand to the receptor exhibited positive cooperativity (ligand binding at one site increases the affinity for ligand at another binding site on the receptor; l>1). Positive cooperativity in the rate-limiting step of protein synthesis (n>1) produced dose-response curves which were "U-shaped" at low doses, indicative of a protective effect provided all other processes were hyperbolic (Hill exponents equal to 1). Positive cooperativity in the metabolism of the xenobiotic ligand (m>1) produced dose-response curves that increased more rapidly than linearity with increasing dose.

The theoretical model clearly illustrates the fact that response cannot be predicted from qualitative mechanistic arguments alone; any assessment of risk to health from xenobiotic chemicals must be based on a detailed quantitative examination of the kinetic behavior of each chemical species individually.

4. Discussion

This exercise demonstrates that we need improved methods for estimating the shape of the

response curve for effects of exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). We created a mathematical model (4) to describe TCDD-mediated alterations in hepatic proteins in the rat. In this model (Figure 2) it was assumed that TCDD mediates increases in the liver concentration of an endogenous ligand of the epidermal growth factor (EGF) receptor, a process which is known to cause internalization of this receptor in hepatocytes. This action is thought to be an early event in the generation of a mitogenic signal. Because TCDD decreases binding of EGF in the livers of intact female rats but not in ovariectomized rats, this effect was further assumed to be dependent on estrogen action. The model also postulates Ah receptor-dependent effects on the concentration of cytochrome P450 1A1 (CYP1A1), P450 1A2 and on the Ah receptor itself.

In a later modeling effort (5), it was found that the expression of message for CYP1A1 seems to follow a model with a Hill exponent greater than 1. This would imply a nonlinear effect on gene expression at low doses and the potential for a threshold-like response. However, this sigmoidal dose-response for message is offset by a supralinear dose-response for protein synthesis on the mRNA template. This leads to proportionate expression of proteing as a function of ligand (TCDD) in the low-dose region.

The net effect of these two exercises implies that there is no apparent reason to believe that gene expression and changes in key proteins following exposure to TCDD will necessarily be linear or non-linear. In general, there is no prescribed form for dose-response curves for receptor mediated carcinogens; each xenobiotic (or congeneric class) must be studied individually if it is to be modeled for dose-response in a mechanistic fashion. The model depicted in Figure 1 can serve as a useful tool in deciding what elements of the biological effects of a xenobiotic ligand will be critical in determining dose-response shape.

5. References

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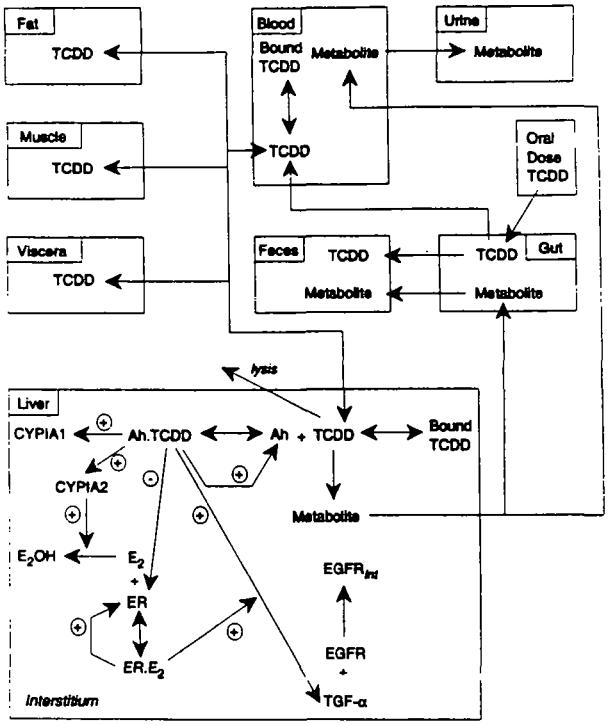

FIG. 1. Flowchart of the NIEHS model of TCDD distribution and consequent effects on gene expression in the rat liver. (Kohn and Portier, 1993)

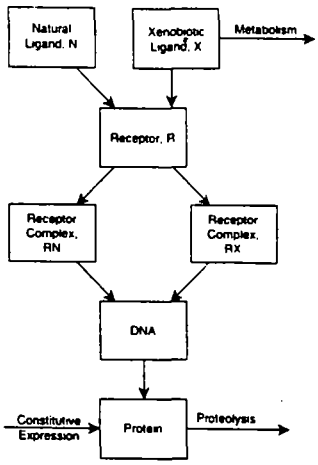

Fig. 2 *Scheme of the theoretical model in this study.* Kohn, et al, 1993)