Neuroendocrinology Letters ISSN 0172–780X

REVIEW ARTICLE

Endocrine Dismodulation and Cancer

Christopher J. Portier

Environmental Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709 USA.

Correspondence to: Dr. Christopher Portier, Ph.D.
NIEHS
PO Box 12233
Mail Drop A3-06
Research Triangle Park, North Carolina, 27709 USA
PHONE:+1 919/541-3802
FAX: +1 919/541-1479
E-MAIL: portier@niehs.nih.gov

Submitted: *May 13, 2002*
Accepted: *May 16, 2002*

Key words: **mathematical model; cancer mechanisms; endocrine disruption; receptor biology; xenobiotic ligand; feedback; crosstalk**

*Neuroendocrinology Letters 2002; **23**(suppl 2):43–47 pii: NEL230802R05 Copyright © Neuroendocrinology Letters 2002*

Abstract

OBJECTIVE: Carcinogenesis has generally been viewed as a genomic disease resulting from genetic mutations occurring at critical locations in the genome in a particular sequence. In the last 10 years, scientists have increasingly identified changes in the levels, frequency and types of endocrine hormones as important contributors to the major cancers faced by western populations such as breast cancer (estrogen, progesterone, prolactin), prostate cancer (estrogen, testosterone), endometrial cancer (estrogen) and thyroid cancer (TSH, T3, T4). This manuscript summarizes cancer mechanisms linked to changes in endocrine function and discusses tools for analyzing and understanding the associated data.

DISCUSSION: A number of chemicals in the environment mimic the role of hormones to bind to receptors (e.g. phytoestrogens as estrogen mimics), alter signaling pathways (e.g. retinoids), inhibit steroid hormone synthesis (such as some fungicides) or alter steroid hormone metabolism (such as TCDD altering the metabolism of both estrogen and thyroid hormones). Genomic and non-genomic endocrine signaling pathways are extensively present in the body and function in a complicated fashion. In order to fully understand the basis for endocrine-induced cancers, one must simultaneously study the various receptors, ligands, enzymes, other proteins within different organs which all contribute to endocrine system function. Also, cross-talk between endocrine systems is common and is key to understanding a potential role of light-dark cycles on human cancer risks.

CONCLUSION: Mechanism-based mathematical models are the only analysis tool available to address all aspects of these complicated networks.

Introduction

According to the Cambridge International Dictionary of English [1], modulate is defined as "to change (something such as an action or a process) to make it more suitable for its situation". Dismodulation refers to the opposite; to change something and make it less suitable for its situation. Endocrine dismodulators (also known as endocrine disruptors, environmental hormones, endocrine active compounds, etc.) are compounds in the environment that are able to change the carefully balanced (over daily, monthly, yearly and life-stage) levels of endogenous hormones in tissues in a living system. Endocrine hormones are produced by certain glands in the body and provide communication between various tissues in the body to regulate a number of critical body functions such as growth, development, reproduction and metabolic homeo-

stasis. The entire family of protein-based hormones consists of approximately 100 small proteins ranging in size from three amino acids (thyrotropin-releasing hormone) to almost 200 amino acids (growth hormones). In addition, a variety of smaller chemical signals, like melatonin, act in the same manner as protein-based hormones. Hormones express their biological action in four different ways; endocrine signaling for communication across different organs, paracrine signaling for communication among adjacent cells, neuroendocrine signaling for synthesis and release of hormones from peptidergic neurons and as neurotransmitters in concert with classic aminergic transmitters. In many cases, a single hormone will have all of these functions. Dismodulation of any of the endocrine systems in the body can result from a growing number of natural and anthropogenic compounds and/or agents with diverse chemical structures and diverse activities.

To discuss the mechanisms through which environmental agents can disrupt endocrine system(s), we must first begin by looking at the minimal structure for an endocrine signaling system. Figure 1 illustrates the five basic components of an endocrine system; a tissue or organ that synthesizes and releases the hormone, a tissue or organ that metabolizes the hormone, a tissue or organ that responds to signals from the hormone, a tissue or organ that controls feedback signals that manage synthesis and release of the hormone and blood or lymphatic tissue to transport the hormone between organs. Not all of these organs need to be unique or independent and any one system could have multiple organs serving the same function. This is an unrealistically simple system in the sense that there is only one feedback (between synthesis and control). However, for the purposes of demonstrating how external factors can alter endocrine systems, this figure can be quite informative.

The five basic components of the simple endocrine system shown in Figure 1 demonstrate the basic targets for endocrine disruption. The next sections describe some of the major ways in which environmental factors can alter an endocrine system. In each case, several examples are given of cancer resulting from endocrine disruption with specific emphasis on changes in the melatonin endocrine signaling system. In addition, analysis methods focusing on the use of mechanism-based mathematical models are discussed to illustrate the utility of these models for addressing hypotheses and in summarizing research findings.

Disruptions in Hormone Synthesis and Release

The precursor peptides that are involved in hormone synthesis follow the classic paradigm of transcription, post-transcriptional modification, translation and post-translational modification in their synthesis. The major disruption that could occur in this compartment of an endocrine system would have to do with mutations in the genes controlling message formulation, mutations in genes controlling post-translational modifications of the precursor peptide or environmental agents that bind to the promoter regions of these genes and either stimulate the production of message (agonists) or ineffectively bind to the promoter region blocking the natural ligand and reduce production of message (antagonist). While there are examples of germline mutations in genes controlling the production of hormones that have health consequences (e.g. a mutation in the gene for the anti-mullerian hormone can lead to bilateral cryptorchidism)[2], it is unlikely that a somatic mutation in any of these genes will have a similar effect without a clonal expansion of the cells carrying the mutation; no such examples exist in the literature. However, there are examples in which

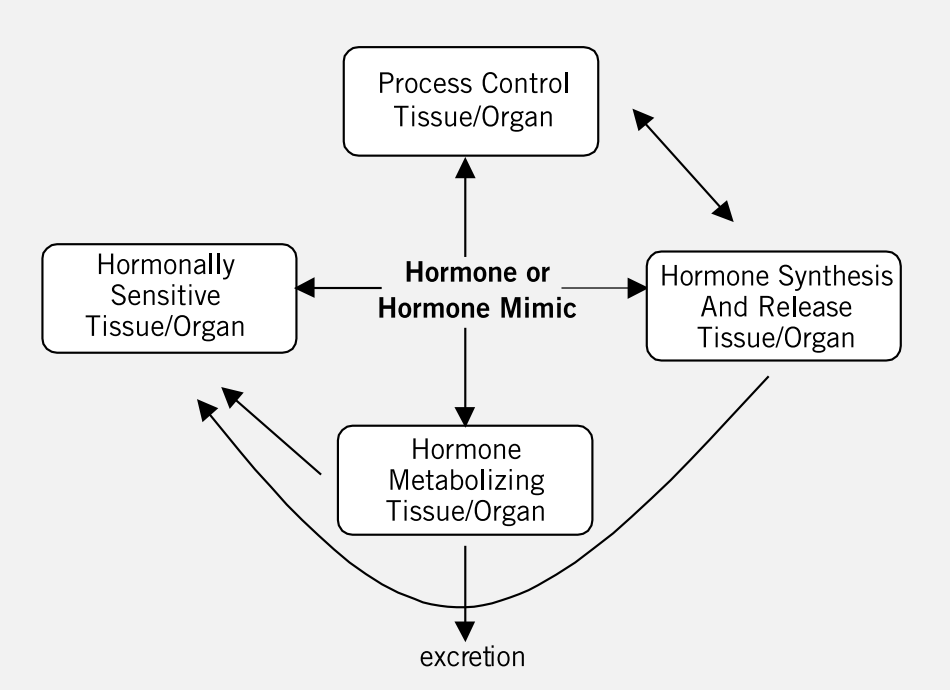

Figure 1. The basic elements of an endocrine signaling pathway

xenobiotic agents can change the expression of either the gene controlling production of the hormone or the enzymes associated with post-translational modification of the hormone.

Melatonin is a biogenic amine secreted by the pineal gland in humans. Unlike polypeptide hormones, melatonin is synthesized through the hydroxylation, carboxylation and acetylation of tryptophan within the parenchymal cells of the pineal gland. Other sources for melatonin exist within the body [3], but the exact location of these sources is still largely unknown. A number of compounds (e.g. okadaic acid, calyculin A,[4] and copper [5]) have been shown to modify the level of the four key enzymes (tryptophan hydroxylase, aromatic-L-amino acid decarboxylase, serotonin N-acetyl transferase and hydroxyindole-O-methyltransferase) that transform tryptophan into melatonin in the pineal gland.

Disruptions in Hormone Metabolism

Like most biochemicals in the body, there is a homeostasis between the production of hormones and their removal via their primary activity (e.g. receptor binding) and/or via their metabolism. Modifications in the metabolism of hormones can occur through mutations in the key enzymes associated with this metabolism or through direct and indirect changes in the level of the key enzymes in metabolizing tissues and organs (e.g. the liver). As in hormone synthesis, it is unlikely that somatic mutations in genes that control the enzymes for hormone metabolism will have a large effect on hormone levels unless the mutated cells clonally expand and replace normal cells. However, much of the work that has focused on gene-environment interactions has demonstrated the importance of germline mutations in metabolizing genes. For example, the high-activity Val432 allele of the CYP1B1 gene [6], which may be linked to oxidative stress through elevated 4-hydroxylated catechol estrogen formation, was associated with an increased risk of ovarian cancer [7]. Changes in the expression of the CYP1B1 gene will alter levels of circulating estrogen; xenobiotics have been shown to both up-regulate the gene (e.g. dioxin) [8]and down-regulate the gene (e.g. 12-O-tetradecanoylphorbol-13-acetate can block the increase induced by dioxin)[9]. Because many tumors are already linked to lifetime levels of circulating estrogens (e.g. breast cancer)[10], changes in these levels are likely to affect the overall cancer risk.

Melatonin produced by the pineal gland of mammals appears in the blood and different tissues and is metabolized by the liver to 6-hydroxymelatonin (predominantly by CYP1A2 [11] with other p-450s playing a minor role)[12], conjugated, and excreted into the urine as 6-sulphatoxymelatonin and 6-glucuronylmelatonin [13]. Numerous agents modify the expression of CYP1A2 and some of these have been shown to affect the levels of circulating melatonin in mammals such as phenobarbital, 7,12-dimethylbenz{a}anthracene, and 17 beta-estradiol [14], furafylline [11] and 2,3,7,8-tetrachlorodibenzo-p-dioxin [15]. As with any change in enzymatic activity, these changes could be due to changes in gene expression for the enzyme or competitive binding of the substrate to the enzyme, blocking its ability to metabolize melatonin. Unlike the metabolic products of estrogen which are DNA reactive, it is unlikely that the metabolites of melatonin increase cancer risks as they are unlikely to be DNA reactive; no literature exists on this topic.

Disruptions in Hormone Response

The primary target for most research into the carcinogenic effects of hormones has been on the tissues that are regulated by the hormone. In most cases, since the presumed mechanism of carcinogenic response from exposure to hormones, hormone mimics or hormone antagonists is controlled through receptor mediated pathways, the binding affinity of the hormone or hormone mimic for the targeted receptor in the target tissue has received considerable attention. *In-vitro* assays exist to evaluate the competitive binding affinity of many agents for important hormone receptors such as the estrogen receptors [16] and the androgen receptor [17]. Disruptions in the ability of the natural ligand for these receptors to bind can increase [18] or decrease [19] the incidence of cancer depending upon the tissue and the particular cancer type. In addition, modification in the ability of cofactors to bind to the liganded receptor can alter the overall carcinogenic response. A similar response can be seen when agents, through transcriptional regulation or post-transcriptional modification, up-regulate or down-regulate the expression of the receptor, cofactors or other protein moieties critical to the activation of hormonal pathways.

It is not certain that decreases or increases in binding to the melatonin receptors will have a direct impact on the initiation, promotion or progression of cancer. However, it is clear that in some cells, the melatonin receptor links to critical pathways associated with cellular replication [20, 21] and apoptosis [22–24], both of which can play important roles in tumor incidence. In some cases, regulation is through combinations of exposures and receptor cross-talk making the interpretation of the effect difficult to evaluate. It is also clear that agents other than melatonin are able to bind to these receptors with differing affinities and differing activities [25–27].

Finally, in many endocrine systems, binding of the natural hormone to its receptor or the cascade of events resulting from this binding serves as a feedback mechanism to control the synthesis and release of additional hormone. It is possible that antagonists could stimulate this feedback mechanism but not activate the receptor for other activity. Such a feedback would reduce circulating levels of the endogenous hormone while simultaneously reducing the availability of receptors available for binding. Such a negative feedback loop could increase cancer risks in some systems like the estrogen cycle.

Analysis of Endocrine Disruption

The complexities of endocrine signaling pathways make analyses using standard statistical methods of analysis difficult to apply in most cases. The usual methods of statistical analysis are based upon simple models describing mean behaviour, such as linear functions or transformations that result in linear functions, and focus

on the analysis of single experiments or replications of experiments. The data describing the myriad of components defining the actions of an endocrine signaling pathway are complex and generally arise from a large series of experiments ranging from *in-vivo* bioassays to *in-vitro* studies of cellular signaling pathways. In order to fully define the impact of an endocrine disruption on overall cancer risks, it is preferable to combine the information from as many of these assays as possible into a single analysis based upon an underlying theory describing the biological mechanisms involved.

Mechanism-based mathematical modeling [28] using sound statistical methods [29, 30] provides a powerful tool for challenging mechanistic hypotheses and for designing studies to improve the strength-of-the-evidence supporting an increased cancer risk in humans from endocrine disruption. Several mechanism-based mathematical models already exist in the literature describing some of the key endocrine systems such as the estrus cycle [31] and the thyroid hormones [32] as well as melatonin [33]. Models describing the pharmacodynamics of receptor binding and activation are also available [34] and can be linked with models of cellular dynamics and control [35, 36] to develop combined models for both analysis and prediction of risks. Through such combined analyses, it is possible to both improve the scientific basis of our understanding of chemically-induced carcinogenesis and to improve the quantification of risks; two examples of where this approach has worked are for dioxin [37] and 1,3-butadiene [38].

In the case of melatonin, there are clear examples of where relatively modest effort in modeling can produce a fairly complex framework for the analysis of potential cancer risks. Blumenthal, Kohn and Portier [33] developed models describing the synthesis, release, distribution and metabolism of melatonin in both rodents and humans. The production and release of melatonin in the model is linked to light-dark cycles through norepinephrine signals sent by the superchiasmic nucleus. New research on changes in light-dark cycle and circulating melatonin levels can easily be accommodated in this model and hypotheses concerning modifications in the synthesis, release, distribution and metabolism can be readily tested and, if necessary, the model can be readily expanded to include new theories linked to recent findings. *In-vitro* experiments showing dose-response for protection against DNA damage by melatonin can be linked directly to in-vivo studies through the estimation of tissue concentrations of melatonin estimated by the model. Expanding to other hormone systems is also fairly straight forward. The estrus cycle model of Andersen et al. [31] using a fixed clock to control the stimulation and development of ovarian follicles which drive the synthesis of estrogen. This fixed clock can be replaced by a direct linkage to the melatonin model with the combined model than able to describe the role of light-dark cycles in controlling both hormones. More extensive models of the estrus cycle are being developed [39] that incorporate all of the key hormones controlling the estrus cycle; linkage to a model of this type may help to provide insights into variation in the usual human menstrual cycle and also the possible effects on the menstrual cycle that might occur from prolonged use of melatonin as a pharmaceutical. Direct linkage of this joint light-melatonin-estrogen model to the models of Pike [40] for breast, ovarian and endometrial cancers can provide estimates of changes in cancer risk as a function of changes light-dark cycles. These predictions can then be used to design both animal studies and epidemiological studies.

Summary

It is absolutely clear that changes in endocrine pathways can induce cancer in both experimental animals and in humans. It is equally clear that changes in the light-dark cycle can alter key endocrine pathways, most notably melatonin and estrogen. Mechanism-based mathematical models are designed to provide an analysis tool that can match the complexity of the data supporting a cancer role for light-induced changes in endocrine systems. Modern computing tools, cutting edge biology and past knowledge can be combined through the use of mechanism-based models to provide a convincing argument in support of this linkage. In addition, these analytical tools can readily identify gaps in our understanding of the mechanisms through which light can modify cancer risks and help to focus our research efforts on the most critical experiments.

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