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Potency matters: Thresholds govern endocrine activity

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ABSTRACT

Whether thresholds exist for endocrine active substances and for endocrine disrupting effects of exogenous chemicals has been posed as a question for regulatory policy by the European Union. This question arises from a concern that the endocrine system is too complex to allow estimations of safe levels of exposure to any chemical with potential endocrine activity, and a belief that any such chemical can augment, retard, or disrupt the normal background activity of endogenous hormones. However, vital signaling functions of the endocrine system require it to continuously discriminate the biological information conveyed by potent endogenous hormones from a more concentrated background of structurally similar, endogenous molecules with low hormonal potential. This obligatory ability to discriminate important hormonal signals from background noise can be used to define thresholds for induction of hormonal effects, without which normal physiological functions would be impossible. From such thresholds, safe levels of exposure can be estimated. This brief review highlights how the fundamental principles governing hormonal effects – affinity, efficacy, potency, and mass action – dictate the existence of thresholds and why these principles also define the potential that exogenous chemicals might have to interfere with normal endocrine functioning.

1. Introduction

The European Commission asked DG Environment to develop a definition of and criteria for identification of endocrine disrupting chemicals (EDCs) applicable to several legislative structures, e.g., the plant protection products regulation (Reg. (EC) No 1107/2009), the biocidal products regulation (Reg. (EC) No 528/2012), and REACH (Reg. (EC) 1907/2006. Besides definitions and criteria for EDCs, the Commission intends to answer whether EDC threshold levels can be determined. Stakeholders offer different opinions on this matter and several agencies have responded to these issues. The European Food Safety Authority (EFSA) recommended clarification of issues regarding biological thresholds and the criteria for adversity versus physiological modulation and homeostatic responses (EFSA, 2013). The Swedish Chemicals Agency concluded "...that the decision on whether or not to accept a non-threshold model for EDCs has to be based on considerations of mechanism of action. Thus, the assumption of no threshold may be as valid, or questionable, for EDCs as for genotoxic carcinogens." (KemI, 2013a). The UNEP and WHO (2013) report entitled "State of Science of Endocrine Disrupting Chemicals" concluded that endocrine disruptors produce non-linear dose responses (there referring to non-monotonic dose response curves) and no threshold can be assumed. Similarly, several publications cited in these reports question the existence of thresholds and suggest that no safe dose can be defined for EDCs.

Overall, six primary considerations have been offered to refute safe threshold levels for EDCs (KemI, 2013b): (1) the complexity of the endocrine system; (2) the presence of sensitive developmental stages; (3) long intervals between the exposure event and the appearance of the adverse effect; (4) no threshold of effect for an endocrine disrupting agent added to a hormone system that is already active, where theoretically, one molecule could activate a receptor when adding to background; (5) scientific difficulties that preclude establishing safe exposure levels, especially for human and other populations, and; (6) the scientific uncertainty in predicting endocrine effects and thereby assessing risks of EDCs.

Many of these considerations can be addressed through an understanding of how the normal functioning of the endocrine system relies on fundamental principles of receptor, enzyme, and transport kinetics, upon which the fields of endocrine physiology and pharmacology are built. The fundamental principles of endocrine action dictate the existence of thresholds that determine whether and to what degree any substance – endogenous or exogenous – may affect the endocrine system. Hence, this

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present review intends to address (1) why the endocrine system could not function if thresholds did not exist; (2) how principles of endocrine pharmacology – affinity, efficacy and potency based on mass action (for reviews of receptor, enzyme and transport kinetics, see Matthews, 1993; Kenakin, 2009) – dictate thresholds, and; (3) why all conceivable effects of chemicals acting through or interfering with aspects of endocrine mechanisms that rely on molecular specificity are governed by these basic rules. In short, as has been concluded for other modes of action (MOA), we assert that principles of endocrine pharmacology imply certain "....rate-limiting key events that, if not met, can lead to a threshold for the dose–response, irrespective of the MOA involved." (Boobis et al., 2009).

We attempt here to concisely describe the fundamental principles that make the case for the existence of thresholds in endocrine action, but we specifically do not represent this work as a critical treatment of all related issues or as a comprehensive review of endocrine action. Toward this end, we have cited general textbooks in several places, for two important reasons. First, some concepts would have required intricate explanations if pieced together from the primary literature that established them, thus reducing clarity and brevity. Second, we wish to emphasize that many principles discussed here are sufficiently well established in the field of endocrine pharmacology that they have been taught in standard textbooks for many years up to the present. Finally, although we make the case that thresholds are obligate for endocrine action, we do not attempt to define thresholds for adverse effects, which may be higher than the thresholds at which normal endocrine functioning can be affected due to ADME and other adaptive and protective mechanisms within animals.

2. Elementary review of endocrine pharmacology

The endocrine system provides major physiological controls in animals with critical roles in development, maturation, and maintenance of health through long-term homeostasis. These functions are accomplished through sophisticated chemical signals mediated by substances known as hormones, which are produced in and released from specific cells and transported, often via blood, to target organs or tissues where the hormonal response is produced (Chedrese, 2009). Many different types of hormonal signals are required for the complex functioning of higher mammals and more than five hundred different effector molecules have been identified in humans (Chedrese and Celuch, 2009). Hormones within related classes are usually derived from common precursors and share similar chemical structures, e.g., steroid hormones derived from cholesterol or catecholamine hormones derived from tyrosine (Chedrese, 2009). Structural similarities extend to many common endogenous molecules, including hormone precursors and metabolites and intermediates and end-products of various biochemical pathways (Chedrese and Celuch, 2009).

Typical extracellular concentrations of functionally active hormones are in the range of 10^{-11} to 10^{-9} molar whereas those of structurally similar, non-hormone molecules (e.g., sterols, amino acids, peptides) are in the range of 10^{-5} to 10^{-3} molar (Chedrese and Celuch, 2009; Grannar, 1993). Given this overwhelming presence of structurally similar molecules relative to hormones, the challenge to maintaining a functional and efficient hormone-based communication system is formidable. Normal endocrine functioning requires that target cells efficiently identify and differentiate the various hormones from other molecules that are present in the extracellular fluid at molar excesses of 10^{6}- to 10^{9}- times (Chedrese and Celuch, 2009; Grannar, 1993). Without the ability to clearly distinguish molecules that convey critical physiological information from structurally similar molecules in the body, the endocrine system would be unable to process specific, vital signals amidst a steady roar of biological noise.

The capacity to achieve these distinctions is based on conformational matching of hormones with receptor structures present in target tissues (Chedrese and Celuch, 2009). These matches are highly selective so that only tight structural pairings produce biological effects that convey important information (Chedrese, 2009; Chedrese and Celuch, 2009). Only certain hormones (called "ligands") fit a particular class of hormone receptors with sufficient complementarity to produce receptor-mediated effects (Chedrese and Celuch, 2009).

2.1. Affinity

Affinity is a primary molecular property enabling the endocrine system to communicate vital information to different tissues of the body and to distinguish this information from biological noise. In broad terms, affinity is the strength of the molecular interaction between a receptor and its ligand (Chedrese and Celuch, 2009; Matthews, 1993), conferring a tendency for the molecules to remain associated once contact has occurred. An endogenous hormone has high affinity for its conjugate receptor such that when contact occurs, a strong molecular interaction follows. Conversely, molecules with low affinity for a hormone receptor will not associate tightly and will more readily dissociate from it.

Affinity has two important consequences for hormone action. A high-affinity ligand fits the receptor well, such that any given contact event is likely to result in a conformationally correct association. This accomplishes the first step of hormone action at the target cell, called receptor binding. Second, for a given number of molecular contact events, a high affinity ligand has a much greater tendency to remain associated with its receptor than a low affinity ligand, a property typically quantified by a dissociation constant. The affinity of a ligand for its receptor determines the fraction of available receptors that will be occupied at any particular ligand concentration (Chedrese and Celuch, 2009; Matthews, 1993), usually referred to as "receptor occupancy." Thus, the greater the affinity, the lower the concentration of the ligand required to bind and occupy receptors.

The affinities of various hormone receptor-ligand combinations can vary depending on the needs of the particular hormonal pathway. Normally, affinities are finely matched with the concentration of hormones required to produce the desired response in target cells (Chedrese and Celuch, 2009). As well, the fraction of available receptors that must be activated to produce a cellular response varies with target cell and tissue type. Overall, affinity dictates whether the ligand has the opportunity to accomplish the second task of hormone action, receptor activation (Chedrese and Celuch, 2009; Matthews, 1993).

2.2. Efficacy

The degree of receptor binding and occupation achieved by low concentrations of high affinity endogenous hormone ligands could theoretically be augmented by a proportionately greater concentration of low affinity ligands, and thus, might lead to cellular responses. However, affinity is not the only determinant of how effectively a ligand activates a receptor. The ability of a bound ligand to efficiently activate a receptor and trigger a cellular response is called "efficacy." There are several theories on the molecular nature of efficacy, including receptor occupancy theory and conformational models, with contributions from post-receptor events (Clarke and Bond, 1998; Kenakin, 2004). Efficacy can range