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Phase I to II cross-induction of xenobiotic metabolizing enzymes: A feedforward control mechanism for potential hormetic responses

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ABSTRACT

Hormetic responses to xenobiotic exposure likely occur as a result of overcompensation by the homeostatic control systems operating in biological organisms. However, the mechanisms underlying overcompensation that leads to hormesis are still unclear. A well-known homeostatic circuit in the cell is the gene induction network comprising phase I, II and III metabolizing enzymes, which are responsible for xenobiotic detoxification, and in many cases, bioactivation. By formulating a differential equation-based computational model, we investigated in this study whether hormesis can arise from the operation of this gene/enzyme network. The model consists of two feedback and one feedforward controls. With the phase I negative feedback control, xenobiotic X activates nuclear receptors to induce cytochrome P450 enzyme, which bioactivates X into a reactive metabolite X′. With the phase II negative feedback control, X′ activates transcription factor Nrf2 to induce phase II enzymes such as glutathione S-transferase and glutamate cysteine ligase, etc., which participate in a set of reactions that lead to the metabolism of X′ into a less toxic conjugate X″. The feedforward control involves phase I to II cross-induction, in which the parent chemical X can also induce phase II enzymes directly through the nuclear receptor and indirectly through transcriptionally upregulating Nrf2. As a result of the active feedforward control, a steady-state hormetic relationship readily arises between the concentrations of the reactive metabolite X′ and the extracellular parent chemical X to which the cell is exposed. The shape of dose–response evolves over time from initially monotonically increasing to J-shaped at the final steady state—a temporal sequence consistent with adaptation-mediated hormesis. The magnitude of the hormetic response is enhanced by increases in the feedforward gain, but attenuated by increases in the bioactivation or phase II feedback loop gains. Our study suggests a possibly common mechanism for the hormetic responses observed with many mutagens/carcinogens whose activities require bioactivation by phase I enzymes. Feedforward control, often operating in combination with negative feedback regulation in a homeostatic system, may be a general control theme responsible for steady-state hormesis.

Introduction

Hormesis is defined as a biological dose–response that exhibits nonmonotonic behavior. At low doses, the endpoint response either increases or decreases from the baseline level; at high doses, the response changes its direction, forming a U- or inverted U-shaped curve (Calabrese et al., 2007). While nonmonotonic biological response may have diverse mechanistic bases (Conolly and Lutz, 2004), hormesis is believed to occur as a result of adaptation of a biological system to stressor-imposed perturbations (Stebbing, 2003; Calabrese, 2008). To ensure robust biological functions at various levels of their organization, living organisms are equipped with a variety of homeostatic defense mechanisms that are activated under stressful conditions to compensate for the undesirable perturbations (Kitano, 2004). At low stressor doses, the compensatory mechanism may overreact to some extent, resulting in a net response that is opposite to the change initially brought about by the stressor; at higher doses, the compensatory mechanism is overwhelmed, leading to a reversal of the response (Calabrese, 2001). Despite the straightforwardness of this overcompensation hypothesis, control systems underlying homeostasis and adaptive response that can result in overcompensation at low doses remain poorly understood. The lack of detailed mechanistic understanding contributes, at least in part, to the current reluctance in adopting hormesis as an alternative risk assessment model, despite that the number of reports on hormesis has grown considerably. Use of mathematical models should help to uncover the operating principles employed by homeostatic control networks and to gain insight into the structural and parametric conditions that can give rise to hormetic responses.

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Hormetic responses occur primarily under two exposure scenarios (Calabrese et al., 2007). In the first scenario, the biological system is continuously exposed to a relatively constant dose of a chemical or stressor of other types. The exposure is long enough that by the time the endpoint response is recorded the system is believed to have reached a steady state. This steady-state hormesis can be found in examples of mutagenesis and carcinogenesis induced by long-term exposure to a variety of chemicals (Camurri et al., 1983; Kitano et al., 1998; Masuda et al., 2001; Sukata et al., 2002; Kinoshita et al., 2003; Kushida et al., 2005; Puatanachokchai et al., 2006). Another frequently adopted exposure scenario involves two sequential dosing events—a priming or conditioning dose is followed by a fixed second dose, with the final overall response evaluated against the conditioning dose (Murry et al., 1986; Ikonomidis et al., 1997; de Mendonca et al., 2000; Koti et al., 2003; Fan et al., 2005; Tang et al., 2005). Hormetic effects arising from this scenario are referred to as conditioning hormesis. Given that homeostatic control networks, including those defending against cellular stresses, are primarily negative feedback mediated (Houk, 1988; Zhang and Andersen, 2007), it is tempting to ask whether activation of this type of network can result in hormesis. In theory, negative feedback control alone may explain conditioning hormesis, as the compensatory mechanism can be activated by the conditioning dose to help moderate perturbations caused by subsequent exposures. However, negative feedback control, be it proportional or integral, is not expected to produce steady-state hormesis, because overcompensation is impossible to occur in theory when such a control system settles to a steady state. Steady-state hormesis is believed to have more bearings to human exposure to environmental toxicants, a situation usually characterized by chronic contact with a toxicant, often at low doses. Therefore, it is imperative to study homeostatic control networks that are responsible for steady-state hormesis.

An important and common homeostatic system inside the cell is the phase I, II, and III families of xenobiotic metabolizing enzymes (XMEs) that control the intracellular levels of xenobiotics and their metabolites. This system consists of an array of enzymes that metabolize xenobiotics via various reactions and eventually export these metabolites from the cell (Xu et al., 2005; Nakata et al., 2006). With phase I negative feedback control, an xenobiotic may activate xenosensor molecules such as aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR), or pregnane X receptor (PXR) to induce phase I enzymes including cytochrome P450 enzymes (CYP), which metabolize the parent chemical into an intermediate metabolite. With phase II negative feedback control, the metabolite may activate transcription factor Nrf2 to induce the so-called phase II enzymes such as glutathione S-transferase (GST), glutamate cysteine ligase (GCL), UDP-glucuronosyltransferase (UGT), etc. These enzymes participate in a set of reactions that add hydrophilic conjugates to the metabolite. Phase III enzymes, most of which are membrane-residing transporters and regulated at least in part by Nrf2, export the conjugated metabolite from the cell. Together, the phase I, II, and III XME detoxification system controls the amounts of xenobiotics and their metabolites that can accumulate in the cell, restricting their downstream toxicity.

Although the primary function of the phase I, II, and III XMEs is to detoxify and eliminate xenobiotics, some xenobiotics are activated in these processes, mainly via metabolism by phase I enzymes. It is estimated that about 3/4 carcinogens are actually XME-bioactivated products from parent procarcinogens (Nebert and Dalton, 2006). Bioactivation by phase I enzymes often converts the xenobiotics into reactive metabolites, many of which are electrophilic and can undergo redox cycling to produce free radicals. These metabolites can react with DNA, protein, and lipids. DNA modification and damage may lead to mutation and carcinogenesis. However, electrophiles and reactive oxygen species are also produced during normal cell metabolism, and in some cases, from background exposure to environmental chemicals, thus establishing a baseline of reactive chemicals in the cell. Since the pool of reactive metabolites, derived both endogenously and exogenously, could be potentially harmful to cellular health, their concentrations need to be tightly controlled.

Homeostatic regulation of reactive metabolites is controlled primarily through negative feedback mediated by phase II enzymes that are induced by electrophilic compounds (Zhang and Andersen, 2007). Crosstalk is also present from phase I to phase II and even phase III enzyme activation (Kohle and Bock, 2006, 2007). Specifically, many phase II enzymes including GST, UGT, sulfotransferases (SULT), NADPH-Quinone Oxidoreductase 1 (NQO1), and some phase III multidrug resistance-associated protein transporters (MRP) can be directly upregulated by parent xenobiotics through nuclear receptors such as AhR, CAR, and PXR (Paulson et al., 1990; Favreau and Pickett, 1991; Emi et al., 1996; Yueh et al., 2003; Ma et al., 2004; Maher et al., 2005; Sugatani et al., 2005; Jigorel et al., 2006; Chen et al., 2007). Recently Miao et al. has found that AhR, which is activated by chemicals from the polycyclic aromatic hydrocarbon family, can directly induce Nrf2 by increasing its transcription (Miao et al., 2005). This route of phase II enzyme and Nrf2 activation by parent xenobiotics, independent of the status of their reactive metabolites, constitutes an inhibitory feedforward control for the reactive metabolites coming out of bioactivation by phase I enzymes. The emerging scheme is thus that the reactive metabolites are controlled by both feedback and feedforward processes (Fig. 1). While the

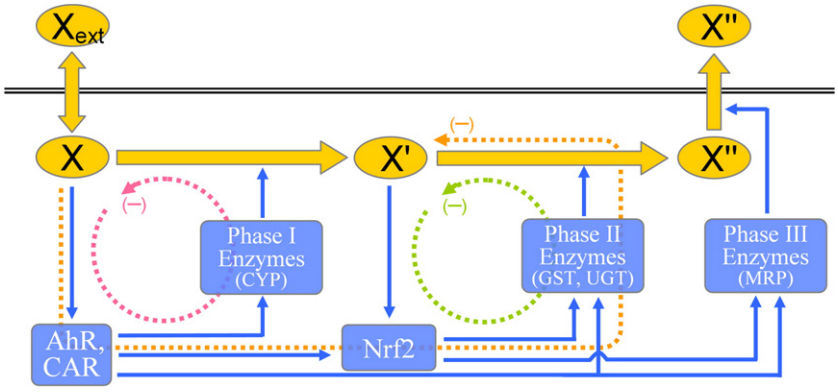

Fig. 1. Schematic representation of the phase I, II, and III xenobiotic control system. Phase I negative feedback (red dashed line) consists of xenobiotic X, nuclear receptors such as AhR, CAR, or PXR, and phase I enzymes such as cytochrome P450 (CYP). The feedback loop functions to increase the metabolism of X and thus reduce its intracellular accumulation. Operation of the feedback loop also results in increased production of metabolite X′ or bioactivation of X if X′ is reactive. Phase II negative feedback (green dashed line) consists of X′, Nrf2, and phase II enzymes such as GST and UGT, etc. The feedback loop functions to increase the metabolism of X′ and thus reduce its undesirable accumulation. Feedforward control via phase I to II cross-induction of phase II enzymes (orange dashed line) consists of X, nuclear receptors, Nrf2 and phase II enzymes. The feedforward control, driven directly by parent chemical X, functions to reduce undesirable accumulation of X′ that could be potentially reactive. X_{ext}: extracellular xenobiotic X; X: intracellular xenobiotic X; X′: reactive metabolite of X; X″: conjugate of X′.