



When normal is not: The dilemma of interpreting laboratory averages of bioactive molecules subject to heterogeneous regulatory feedback and epigenetic mosaicism

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Summary Complex regulatory systems control the levels of many bioactive molecules in the serum. These systems involve the integration of feedback responses from numerous tissues. End-organs and tissues can manifest epigenetic mosaicism, particularly with aging or disease states. We propose that an isolated lab value may reflect a blended average of inhomogeneous feedback responses from target tissues in various states of dysfunction. Reliance on such data may underestimate the state of systemic dysfunction. Yet in clinical practice, normal serum levels of a given molecule and its associated regulatory machinery are often assumed to reflect normal body homeostasis and tissue function. Organism-wide integration of abnormally high and low levels of bioactivity of a molecule in different tissues may yield apparently normal serum values of the bioactive molecule and known components of its regulatory system. We specifically discuss thyroid hormone regulation and function as a case example. Epigenetic reprogramming of either regulatory loops or tissue responses represents another way in which normal serum levels of the molecule may obscure target-organ dysfunction. The proposed idea has broad implications for disease pathogenesis, diagnosis, and therapies. A model where individual tissues employ illegitimate signaling to subvert the concerns of the organism as a whole is also proposed. © 2005 Elsevier Ltd. All rights reserved.

Introduction

The serum levels of many molecules including electrolytes, hormones, coagulation factors, metals, and binding proteins are subject to complex regulation [1–4]. The precise molecular basis for regulatory control is often not known. However, in

combination with biochemical and molecular data, empiric observations have provided insight into the constituents of some regulatory loops and aspects of their function. For example, neural–pituitary–endocrine organ–target tissue axes regulate many hormone levels. Dietary intake, absorption, tissue uptake, tissue utilization, and serum binding proteins regulate the serum levels of many metals in a combinatorial fashion [5–8]. In many cases, a normal value range has been determined for known

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components of regulatory systems for particular molecules [3,9–12].

In clinical medicine, the assessment of a variety of illnesses requires the quantitative measurement of known constituents of a given molecular pathway. If these levels fall within normal limits, one may assume that the pathway exhibits normal function. Many examples of this prevailing approach exist. To determine if there is an excess or deficiency of selenium, one must both clinically obtain serum levels of this metal and also measure a specific enzymatic activity that depends on selenium [12]. Copper and zinc levels are measured to screen for adequate dietary intake and serum levels [11]. The evaluation of body iron status relies on initial measurement of the serum ferritin and transferrin levels [13], while the evaluation of iron deficiency anemia begins with simply measuring the ferritin level [13–15]. The basis for assessment of many endocrine pathways involves measuring levels of the specific hormone or its immediate upstream control molecule – for instance, thyroid stimulating hormone (TSH) and thyroid hormone [16]. Indeed, one would observe standard medical practice in obtaining serum measurements of a wide variety of molecules. Then, if this array of tests all show findings within normal limits, one traditionally assumes that overall homeostasis remains intact [17].

Hypothesis

We hypothesize that an isolated lab value may reflect a blended average of inhomogeneous feedback responses from target tissues in various states of dysfunction. A fundamental flaw may exist in assuming that normal serum levels of molecules and their regulatory systems are necessarily indicative of normal molecular function. While this assumption may partially apply to many patients, we propose that the traditional approach may overlook a large number of pathologic conditions where dysfunction in individual organs and tissues perturb the function of the molecule.

This model proposes that two major forms of dysfunction can occur in the setting of normal laboratory values. In one instance, a combination of high, normal, and low sensitivities to a specific molecule simultaneously exists for different tissues or tissue regions in a given individual. In this scenario of heterogeneous regulatory feedback, different cells and tissues produce signals corresponding to both high and low feedback, but the cumulative signal sums to a normal value and therefore generates normal levels of the molecule.

If the feedback signals from tissues are variable, an abnormal value may also incorrectly register the degree of dysfunction. In a second variation, epigenetic reprogramming of either regulatory loops or tissue responses occurs. In this case, serum levels of the molecule and the activities of its control systems are normal, but individual tissues have abnormal sensitivities to the molecule. We use the term “epigenetic mosaicism” to describe this phenomenon.

Thyroid hormone as an index example

The current standard of practice for the evaluation of thyroid dysfunction provides a specific example to highlight this concept. Screening for abnormalities in thyroid function is frequently accomplished with a single test – the measurement of TSH [18]. If TSH exists within normal levels, one presumes the thyroid hormone loop to be functioning normally. The validity of this assumption comes from clinical data demonstrating that the regulatory control loop between thyroid hormone levels and TSH is robust [19], despite the complex regulation of the peripheral metabolism of the different forms of thyroid hormone [20–22].

In our model, various tissues within an individual can exhibit different sensitivities or responses to thyroid hormone, such that the net level of circulating thyroid hormone is normal and the TSH is normal. For example, in obesity, peripheral tissue may become relatively refractory to the effects of thyroid hormone, and consequently exhibit decreased peripheral conversion and utilization of thyroid hormone. Such an occurrence would reduce the efficiency of fat store mobilization, creating an ongoing requirement for high levels of caloric intake to support caloric expenditure. Thus, the bowel, another site of thyroid hormone sensitivity [23–26], would remain active and even possibly become hyperactive, therefore continuing to require normal or even supra-normal amounts of thyroid hormone. Likewise, secondary to increased body mass, the heart would become subject to a variety of abnormal stresses with activities of daily living, and may also require normal or even supra-normal levels of thyroid hormone, especially in clinical situations where other compensatory modes, such as sympathetic activation, undergo pharmacologic blunting. Therefore, a hypothetical patient might exhibit obesity, normal to hyperactive bowel function, and excess basal cardiac activity. The net level of thyroid hormone would be normal, but individual organs would have functional hypothyroid or hyperthyroid states. The combination of symptoms would not exemplify global hypo or

hyperthyroidism, but would instead reflect individual organs independently exhibiting features of either hyper or hypothyroid states.

Depression constitutes another disease harboring an association with low levels of thyroid hormone [27], suggesting that normal activity levels and mood require the actions of thyroid hormone. In a similar fashion to obesity, if the brain develops local resistance to the effects of thyroid hormone in a patient with underlying cardiac disease and a heightened cardiac requirement for thyroid hormone, the patient would again exhibit normal thyroid levels in the serum, despite thyroid malfunction as contributing factors to both the depressive symptoms and the cardiac dysfunction.

Although the specific effects of thyroid hormone on target tissues remain complex and not completely understood, deiodinases appear to convert thyroxine (T4) into the more active triiodothyronine (T3) form in various tissues [20]. T3 associates with thyroid receptor (TR) isoforms that have tissue specific expression patterns [28]. Following ligand binding, activated TR participates in a varied array of activities, including regulating gene expression, modulating chromatin structure, as well as directly affecting mitochondrial function [29–33]. Many of the effects of the TR occur in combination with other molecules [34,35]. Numerous possible mechanisms including tissue specific regulation of deiodinase activity, differential expression and activity of receptor isoforms and co-factors, and epigenetic programming might impart tissue specific perturbations to the thyroid hormone response.

Evidence exists that each of these processes can modulate tissue specific responses to thyroid hormone. For example, selective agonism of the beta isoform of TR affects serum lipids without modulating heart rate [36]. The activity of different deiodinases can generate T3 with bioavailability either in specific tissues or on a serum-wide basis. Moreover, tissue specific expression of the D3 deiodinase catalyzes the conversion of T3 to the inactive rT3 [20]. Expression of the D2 deiodinase involves post-transcriptional control, and overexpression of D2 in cardiac muscle causes localized thyrotoxic effects in mouse models [37]. Therefore, any tissue specific change in post-transcriptional regulation of deiodinases could induce tissue specific modulation of thyroid activity. Wassen et al. [38] showed that D3 activity increased in rat hypertrophic heart failure models, suggesting that cardiac specific modulation of deiodinase activity may contribute to disease phenotypes. In addition, Kinugawa et al. [39] correlated cardiac specific “cellular hypothyroidism” in rat heart

failure with changed expression of TR isoform expression in cardiac dysfunction. In human patients with hypothyroidism, levothyroxine therapy appeared to occasionally associate with a discrepancy between certain markers of thyroid activity and TSH levels [40]. However, none of these studies explored the possibility of a general mechanism whereby relative hypo or hyperthyroidism induces disease in the setting of normal levels of TSH.

Organism-wide heterogeneity in response to bioactive molecules

Evidence also exists for other molecules and pathways in support of the proposed hypothesis. Although the broad format suggested here has not previously been proposed, individual examples of variable tissue processing and responses to various molecules and pathways have been previously identified.

For example, resistance to the effects of insulin can develop in a variety of tissues, with resistance in skeletal muscle and adipose tissue believed to be critical in the development of diabetes [41–43]. How a given tissue develops resistance to insulin remains unclear, although insulin resistance seems to play a role in pathology in a wide variety of tissues, even in the absence of overt diabetes [44]. For instance, considerable heterogeneity exists in the development of non-alcoholic fatty liver disease and its association with insulin resistance. Specifically, many diabetics do not have fatty liver disease, and fatty liver disease can also occur in non-diabetics [45–47].

Diabetics may also have dramatically varied resistance to insulin in different tissues. This model may explain why tight diabetic control does not eliminate all diabetic complications, since localized defects in insulin function may occur in diabetics even in the setting of normal organism-wide glucose homeostasis. Normal levels of insulin and glucose do not always appear to reflect normal glucose and insulin homeostasis.

Glucocorticoids represent another hormone class that supports the proposed model, Stewart notes that Cushing’s syndrome (glucocorticoid excess) is associated with obesity, hypertension and osteoporosis [48]. Attempts to identify cortisol excess as an isolated cause of these diseases led to the discovery that the 11β -hydroxysteroid dehydrogenase acts at the tissue level in controlling the exact concentration of cortisol that is available to bind to the glucocorticoid receptor. Tissue specific perturbations in the activity of these dehydrogenases apparently lead to “tissue-specific Cushing’s syndrome”. Although this research did not address

a mechanism of action independent from that of the hypothalamic–pituitary–adrenal (HPA) axis [49], Rask et al. [50] suggested a potential mechanism by demonstrating increased liver-based metabolism of cortisol in obese patients. These patients also had increased local adipose tissue concentrations of 11 β -hydroxysteroid dehydrogenase (11 β -HSD) Type 1, which increases local adipose concentrations of active cortisol. We believe that the 11 β -hydroxysteroid dehydrogenase Type 1 story represents just one supporting example of a potential host of tissue specific mechanisms through which cortisol activity can be altered at the cellular level with remaining evidence of a normal HPA axis. Specifically, we would anticipate that epigenetic alterations in the HPA axis may occur that leave ACTH and cortisol levels unchanged despite tissue-specific relative cortisol excess or deficiency. We also would anticipate that tissue-specific mechanisms in addition to 11 β -HSD Type 1 modulation may occur so that organism-wide cortisol activity sums to normal despite a wide range of cortisol function in individual tissues.

Iron affords a non-hormonal example of our hypothesis. Dietary iron and serum ferritin levels both influence the regulation of iron absorption by modulating dietary iron uptake from the gut [5,6]. However, the serum ferritin level does not always reflect the level of gut absorption or total body iron stores. In hemochromatosis, an inappropriate regulation of gut absorption of iron leads to excess absorption and storage of iron [51]. In liver disease, storage of iron in the liver does not necessarily manifest in the serum ferritin level [52]. Thus, serum ferritin level does not necessarily reflect total body iron levels due to organ specific modulation of iron trafficking. Similar dysfunctions in feedback and storage loops may occur with regard to the trafficking of other dietary elements.

Selfish organs and a desire for autonomy as a contributing cause

From an evolutionary perspective, a major challenge during the development of multi-cellular organisms involved subverting the actions of individual cells that would otherwise seek benefit for themselves as opposed to benefit for the organism as a whole. In more complex organisms, overarching systems of control supervise the actions of aggregates of cells that have shared function – tissues and organs – in order to maximally utilize their efforts for the greater benefit of the organism. Cancer represents the classic example of a disease of inappropriate cellular autonomy that afflicts complex

organisms. Research has documented extensive programming both within individual cells and on an organism-wide basis that intends to prevent the development of this particular form of cellular autonomy. Such safeguards include sophisticated DNA damage repair machines [53], cellular suicide programs, default differentiation pathways, immune surveillance, and neuroendocrine modulation of cellular behavior.

Cancer likely does not constitute the only type of transformation to generate selfish cells or tissues. A large number of control mechanisms, analogous to cancer prevention machinery, serve to regulate numerous cellular and organ functions. In our model, just as malignancies may evade the efforts of cancer prevention machinery, cells and tissues may also escape other schemes of regulatory control. Energy acquisition and resource utilization represents one area that likely underwent intense selective pressure in the unicellular past. One would predict that inappropriate acquisition or utilization of energy would represent a dysfunction that may lead to autonomous behavior on the part of a cell or tissue that proves deleterious to the organism as a whole. Likewise, uptake, utilization, and secretion of trace elements play an important role in unicellular life. Inappropriate utilization of trace metals might also develop on a cell autonomous basis in multi-cellular organisms.

On the level of single cells, mutation, epigenetic reprogramming, or random drift of cellular machinery may explain how cells bypass control mechanisms that normally ensure that cellular activities benefit the whole organism. Clones of cells could arise through stable propagation of these events [54,55]. Global factors may disrupt organism-wide control mechanisms such that entire organs become inappropriately regulated with regard to optimal homeostasis of the whole. Fat storage provides one example where circadian and seasonal rhythms likely participated in programming of storage and utilization patterns. As these signals have become lost in modernity, maladaptive drift may occur throughout adipose tissue. Thyroid dysfunction in adipose tissue may represent one manifestation of this dysfunction. Numerous other potential regulatory programs may also stray and become dysfunctional in the modern world.

More localized factors could also contribute to maladaptive autonomous behavior of cells or tissues, including local trauma, scarring, and mechanical forces, such as shear stresses in hypertension. Tissues near localized perturbations may have a greater tendency to develop autonomous behavior,

much as tissue injury and inflammation has been theorized to predispose to malignant transformation [56]. In addition, some organs may provoke systemic neurohormonal activation via local release of soluble factors or electrochemical activity so as to induce the autonomic nervous system or the HPA axis. Other organs may then attempt to counterbalance the systemic modulation through their own unique responses, potentially leading to a situation where different organs develop apparently contradictory or paradoxical behaviors.

If individual cells or groups of cells develop partial autonomy with respect to a metabolic function or utilization of a scarce resource (such as a trace metal), one would expect other cells, organs and tissues to develop compensatory changes as the organism struggles to maintain homeostasis. As individual tissues and organs drift further from normal homeostasis, diverse phenotypes that we would ultimately recognize as diseases would eventually become evident. The underlying etiology of these diseases may be traced to a distant organ or tissue unrelated to the site of primary manifestation.

Implications

Modern technology is uniquely poised to verify or disprove the generality of this theory. If major regional variation in the utilization of serum molecules occurs, the need to develop tissue specific diagnostic tests for a variety of conditions becomes readily apparent. A potential solution to detect epigenetic mosaicism may involve molecular imaging techniques, such as tagged radionuclide scans to quantify the degree of heterogeneity in the feedback system. From a therapeutic standpoint, in some cases restoration of normal organism-wide homeostasis may require the use of combinations of agonists and antagonists for a given pathway. Alternatively, sequestered local delivery of treatments may prove critical for correcting local derangements of systemic pathways or for enabling treatment of different organ comorbidities which may require the simultaneous application of agents with opposite actions. For example, we envision the possibility that an array of separate peripheral neuromodulation devices which correct independent end-organ dysfunctions at the local level might be coordinated by a central intelligent circuit. An important role may exist for epigenetic reprogramming through either pharmacologic or dietary manipulations. Future research using genomics, transcriptome analysis, and proteomics may indeed validate our line of reasoning.

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