Most biological outcomes such as blood pressure, glycemia, appetite, and body weight are influenced by a number of interacting factors or mechanisms. This characteristic is captured in a number of classical endocrine concepts, including integration, coactivation and regulation, redundancy, hierarchy, and synergy. Whereas the usefulness of most of these concepts lies mainly in their heuristic value and qualitative aspect, synergy is also often used in a quantitative way, to describe interactions of hormones, drugs, or other manipulations that are “supra-additive.” We are basically told that $1 + 1 > 2$. The questions thus arise: is superadditive synergy meaningful, how is it measured, and how does it work? A clear example of superadditive synergy can be found in catalytic chemistry, when a very small amount (dose) of a catalyst that by itself cannot produce an effect drastically amplifies a chemical reaction. But do similar synergies exist in endocrinology and physiology, in which 2 or more biological agents, often with very different molecular mechanisms and temporal dynamics, can affect specific outcomes or responses? It seems logical that interfering simultaneously with several interacting mechanisms would have a larger effect on the outcome measure than targeting just one. Using multiple drugs to improve BP or glucose homeostasis is already common practice, and the strategy is increasingly exploited in new combination therapies for obesity and other multifactorial diseases. For example, several antiobesity drugs combining 2 or even 3 receptor domains with the same molecule are currently under development (1–3).

Because supra-additive synergy is an old concept in pharmacology, most researchers probably believe that it rests on a well-established theory that is readily transferable to endocrinology. Closer inspection suggests that this is not the case. One set of problems concerns the applicability of the concept to complex integrative biological systems. Another issue concerns quantification. A notion of supra-additive synergy obviously depends on some form of addition. As a recent critical review by Geary (4) indicates, the classical additivity metrics are neither straightforward nor clearly valid. This comment briefly describes both problems with existing approaches to supra-additive synergy and an alternative approach.

**Biological Difficulties**

Clearly, supra-additive synergy depends on identifying what to add. The choice is not obvious. For example, one classical approach is based on addition of effects, and another on addition of doses (discussed in the next section). There is, however, a wider problem. Mathematical synergy models are based either on purely functional input-output models (eg, the probabilistic model of Bliss (5) and the isobolographic model of Berenbaum (6) or on classical receptor-ligand interaction models [eg, Refs. 7–9]). These approaches seem overly simplistic in that they fail to capture the biological complexity underlying endocrine function and, therefore, may divert attention from it.

The actions of gonadal steroid hormones amply illustrate many of the inherent biological complexities of endocrine systems that highlight inadequacies of present synergy analyses. Consider an interaction experiment involving administration of estradiol and some other agent. An initial problem is that even if one gonadectomizes the subjects in order to reduce endogenous estrogens, there may still be steroidogenesis in target tissues, including the adipose tissue and brain, leading to synthesis of androgens and progestins as well as estrogens (ie, estrone, estriol, estradiol, and perhaps neurosteroids with estrogenic activity, all of which have distinct estrogenic...
activities [10–13]). Thus, the pattern of both local and endocrine-mediated steroidal stimulation will not be precisely controlled. Rather, exogenous estradiol will interact with a dynamic endocrine background, the effects of which will be difficult to distinguish from synergy per se. Another consequence of such background effects is that they make impossible precise threshold determinations, which are often used in synergy analyses. Receptor multiplicity and dynamism lead to similar problems. Estrogens act on 2 classical receptors, α and β, which are present both in the nucleus as well as the cell membrane, as well as on other, novel membrane estrogen receptors (14–16). The relative quantities of these different receptors are labile; for example, they are affected by gonadectomy. Again, therefore, altered responses may reflect recruitment of different mechanisms rather than synergy in a single mechanism.

A final example relates to temporal dynamics. Both early developmental and later effects of estrogens depend upon stimulation of estrogen receptors in a particular temporal sequence (eg, Ref. 17). For example, maximal facilitation of lordosis, a reflexive preceptive behavior displayed by female rats, depends on distinct estrogenic feedbacks to the hypothalamus at 2 distinct time points (18). Thus, estrogens synergize with themselves. This is another characteristic endocrine effect that is not captured by synergy models designed to describe the simultaneous application of different agents. Estrogen signaling is by no means unique in these respects, and neurotransmitter systems as well as other endocrine systems display similar dynamisms. These considerations question whether supra-additive synergy is a helpful concept in the analysis of endocrine interactions. Is a slightly subadditive interaction less interesting than a supra-additive interaction, and does it necessarily result from a qualitatively different mechanism?

Quantitative Difficulties

Supra-additive synergy analyses depend upon comparing the interaction effect to a prediction based on addition of the individual effects. Various strategies for computing such additive predictions exist, but the underlying theory and assumptions are rarely examined. Geary (4) has now done this in a stimulating and provocative recent article entitled, “Understanding Synergy” that should be of particular interest for the readers of Endocrinology. In a nutshell, after careful evaluation of the theoretical and mathematical bases and combing through more than 170 references, many in this journal, he argues persuasively that almost all supra-additive synergy analyses lack validity; the emperor is not wearing clothes! The problems, in Geary’s view, are so intractable that supra-additive synergy should be abandoned in favor of a simpler approach that does not rely on additive predictions but rather rests on defining synergy as simply an increase in effect. His message echoes the conclusions already reached half a century ago by the German pharmacologist Loewe (19) and other scientists since then (20, 21). As recently as a year ago, Shafer (20), analyzing synergy models in anesthesia, concluded that although useful, “all models are wrong.” The problems with supra-additive synergy arise essentially from the fact that additive predictions rely implicitly or explicitly on the types or forms of the dose-effect relations of the agents being tested. Existing approaches are limited to a very restricted set of forms of dose-effect relations. This is true for both of the basic approaches of “response addition” and “dose addition.”

Response addition refers to defining synergy as an interactive effect of doses of 2 drugs, hormones, etc., that exceeds the sum of the individual effects. This is the most common synergy approach. Geary makes the limitations of response addition clear with a “sham addition” thought-experiment, in which the 2 hormones are, in fact, identical. It seems self-evident that the effect of joint application of 2 doses of the same hormone should be additive. In the simplest case, doubling its dose should result in twice as large a response. However, this is not the case when the dose-response relationship is nonlinear. If the relevant part of the dose-response curve is accelerating, doubling the dose will result in a much higher than expected response (Figure 1), and if the curve is decelerating, doubling the dose will result in a lower than expected response. The same problems occur when doses of 2 different drugs or biological agents are added together. Only if the dose-effect curves for both hormones are linear does response additivity give correct results. Because this almost never happens, response additivity is dismissed as almost never valid. This is because in the region of threshold doses, almost all dose-effect curves are accelerating; therefore, the strategy of testing subthreshold doses does not avoid the problem.

The analysis of dose additivity is somewhat more complex, but equally problematic. Most dose-additivity approaches use what is called “Loewe additivity” to generate additive predictions. Loewe additivity is a procedure for transforming the dose-effect curve of one hormone into that of another. One simply posits that each dose of one hormone can be considered identical to the dose of the second hormone that leads to the same effect. Additive predictions are based on summing a dose of the first hormone transformed to the “equivalent dose” of the second hormone and
identifying the effect of that summed dose on the second hormone’s dose-effect curve.

Dose additivity analyses are usually done by plotting all the dose combinations that should lead to a particular specified additive effect on a graph the x-axis of which is the dose of the first hormone and the y-axis is the dose of the other. This plot is known as an “isobolograph.” It is generally assumed that isobolographs are linear and independent of the form of the dose-effect curves of the 2 hormones considered. This assumption is based on the influential work of the synergy theorist Berenbaum (6) about 25 years ago. Geary demonstrates that Berenbaum’s conclusion was based on faulty mathematics and that isobolographs are usually curvilinear (ironically, Loewe, the inventor of “Loewe additivity” recognized this decades ago, but his work is rarely read). The upshot is not only that linear isoboles are very rare (they occur only if the dose-effect curve of one hormone is identical to that of the other multiplied by a constant). Worse, in most cases in which the dose-effect curves are not multiples of each other, Loewe transformations of one hormone into the second give different results than transformations of the second hormone into the first (ie, adding A to B leads to a different prediction than adding B to A!). An example of Loewe transforms leading to such indeterminate additivity is shown in Figure 2. Another problem with isobolograms occurs if the maximum effect of one hormone exceeds that of the other; in this case, the Loewe transformation cannot be done for effects exceeding the lower maximum. In addition, as Geary shows, many variations of dose additivity use different methods to generate additive solutions, but still use the linear isobologram to test whether they are synergistic or not. Thus, like response additivity, dose additivity fails to offer a generally valid approach to supra-additive synergy.

Figure 1. Response additivity can produce erroneous predictions for drugs or biological agents with curvilinear dose-effect relationships. In the example shown, the effect of dose 2 is 1, so that according to the principle of sham combinations, doubling the dose from 2 to 4 would be predicted to yield a response of $2 \times 1 = 2$. In fact, due to the concave-up shape of the dose-effect curve in that range, the observed effect of dose 4 is 5.3, or more than twice as large as predicted. Thus, nondiscerning use of response additivity can erroneously lead to the conclusion that low doses of this drug synergize potently with themselves. Because almost all dose-effect curves are accelerating in the region of threshold doses, the problem is particularly pertinent to response addition of 2 subthreshold doses. Adapted from Geary (4) with permission.

Figure 2. Graphic illustration of the dose-equivalence principle yielding indeterminate Loewe-additive solutions. A, Dose 1 of A is equivalent to dose 3.2 of B (dashed lines), and dose 1 of B is equivalent to dose 0.3 of A (full lines), as explained in the text. B, Note that different additive predictions are generated for combination of doses 1 of A plus 1 of B when the A-equivalent dose of B is used (full lines; 1.3 units of A yields effect of 5.2) than when the B-equivalent dose of A is used (dashed lines; 4.2 units of B yields effect of 6.3) is used. In many cases the disparity is much greater. Adapted from Geary (4) with permission.
What Is the Solution?

As Geary (4) argues, it seems unlikely that a general mathematical approach to replace Loewe additivity will be found, and even if one is found, it will almost certainly lack the intuitive appeal of either simple response additivity or linear isobolograms and, therefore, is unlikely to be sufficiently user friendly to be adopted by most endocrinologists. Therefore, the wisest choice might be to simply report interactions for what they are worth, changes in effect, and not hide behind “voodoo-mathematics” as justification to make additive predictions and claim supra-additive synergy. This simple approach is already popular in drug development, where synergy is defined simply as combinations of drugs that lead to statistically significant increases over the effect of either agent alone. This requires no theory of addition and can be done for individual doses without characterizing a full dose-effect curve. It does not obscure or divert attention from the complex mechanisms underlying endocrine interactions but rather can efficiently direct researchers to the larger of these interactions. Furthermore, an adaptation of the method is to identify drug combinations that lead to the largest difference between therapeutically desirable effects and undesirable side effects. As Geary points out, this form of synergy is now recognized as sufficient for Food and Drug Administration approval of drug combinations. Researchers interested in neural and endocrine interactions should be aware of this evolution of synergy theory.

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