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COMMENTARY

Commentary on Neostigmine Interactions with Non steroidal Anti-inflammatory Drugs by Miranda *et al.*

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The article by Miranda *et al.* presents data showing significant synergism between NEO and certain NSAIDs administered i.p. in the mouse. Their conclusions follow from isobolar analysis accompanied by statistical confidence limits that show significant differences between the additive (expected) and experimental potencies. This commentary discusses the features of the graphical method of analysis and points out other experimental designs and the methods used to analyse them. *British Journal of Pharmacology* (2002) **135,** 1589–1590

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When two drugs that produce overtly similar effects are given together the combination may produce effects of exaggerated intensity. This phenomenon, known as synergism or super-additivity, is important clinically and is also useful for examining mechanism. Several examples of drug-combination studies illuminating mechanism are given by Tallarida (2000; 2001). The demonstration of synergism requires evidence that the potency of the drug combination is greater than that calculated from the individual drug potencies. Toward this end the isobologram, a graph displaying equally effective individual and combination doses, is quite useful when accompanied by an appropriate statistical analysis. The isobologram is amply illustrated in the paper by Miranda *et al.* (this issue) that describes the results of antinociceptive tests with neostigmine (NEO) paired with several different non steroidal anti-inflammatory drugs (NSAIDs). The graph is constructed by plotting the individual drug potencies (e.g., *ED50* or *D50*) as intercepts of a Cartesian coordinate system, thereby defining a downward-sloping line (line of additivity) that creates a triangular region in the first quadrant. The line is the locus of points representing dose pairs that are expected from the individual potencies. The combination dose pair that experimentally gives the same effect is then plotted and, if contained within the triangle, indicates synergism. If the plotted dose pair lies outside this region the combination is termed sub-additive while a point on the line indicates simple additivity.

In this interesting paper the authors point out that the known mechanism of NSAIDs, inhibition of cyclo-oxygenase, is not quite sufficient to explain the analgesic efficacy of these drugs. Acting on previous suggestions that acetylcholine is an endogenous antinociceptive compound, the authors tested pairs containing one of several different NSAIDs each with NEO in the mouse writhing test. They found synergism with certain intraperitoneal dose combinations. This finding, they conclude, adds suggestive evidence of supraspinal antinociceptive modulation resulting from increased acetylcholine concentration in the synaptic cleft of cholinergic interneurons. Readers of their paper may individually judge the merit of the authors' interpretation but all are likely to agree that this kind of study, i.e., employment of a drug combination, can be valuable in illuminating mechanism. The administration of even a single drug to any organism or biological system places it in potential contact with a myriad of endogenous chemicals. Consequently, an experiment with a second chemical that mimics, enhances or otherwise affects an endogenous substance can provide new clues for understanding mechanism. The study by Miranda *et al.*, by its use of NEO, implicates the cholinergics in peripheral and central sites related to inhibition of pain perception, a mechanism postulated by Burkle *et al.* (1998). Equally interesting are those situations in which one of the two drugs has no obvious efficacy, is not an antagonist, but yet acts in association with an efficacious drug to enhance its potency. An example is given by Vaught & Takemori (1979) who reported that i.c.v. [Leu^5]enkephalin did not produce antinociception over a range of doses tested but, nevertheless, could still enhance the potency of morphine. Porecca *et al.* (1990) examined this finding quantitatively by administering [Leu^5]enkephalin i.p. to mice that also received morphine by this same route. They found strong synergism among several fixed-ratio combinations of the two agents. A recent finding by this writer and colleagues (Tallarida *et al.*, 2002) shows that glucosamine, which lacks antinociceptive efficacy in the mouse writhing test, strongly potentiates the antinociceptive effect of ibuprofen. The analysis of data for this kind of situation in which one drug lacks efficacy is straightforward. One need only compare the active drug's dose-response curve before and after addition of the second compound. Such studies stand in contrast to the Miranda study in which both the NSAIDs and NEO displayed efficacy.

Besides these isobolar experimental designs in which doses for matching effects are compared, other designs can be used. One example is response surface analysis that records the effect produced by independent dose pairs and examines the experimental effect in relation to the additive effect which is plotted 3-dimensionally as a surface in Cartesian coordinates (Tallarida, 1999; 2000). This is a newer approach that identifies possibly varying degrees of synergism (as well as additivity or sub-additivity) over all meaningful drug

combinations. Whereas the response surface method is newer than the isobolographic method, the use of either is still relatively rare in the literature. This lack may be due to the absence of these topics in mainstream textbooks, a fact that prompted this writer's decision to produce a monograph on the subject (Tallarida, 2000). An important feature applicable to all aspects of work with agonist drug combination is the requisite statistical accompaniment. The distinction between simple additivity and non-additive interactions requires statistical demonstration. Essentially, this distinction comes down to demonstrating that two quantities (e.g., *ED50*'s) differ significantly. Because *ED50*'s and other potency measures have error this demonstration requires statistics. The study by Miranda *et al.* accomplishes this need with clarity by its use of 95% confidence limits of the theoretically additive and experimental doses. The isobologram alone would not be sufficient in showing such distinctions.

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