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A quantitative study to assess synergistic interactions between urotensin II and angiotensin II

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Abstract

Interaction between the vasoactive peptides, urotensin II and angiotensin II, could have important implications in various disease states. We examined this interaction using isolated rat aortic rings with intact adventitia and endothelium. The fixed-ratio combination we tested produced effect levels significantly greater than predicted by additivity. Thus, the interaction was synergistic, and this is illustrated in a response surface plot that shows the predicted additive effect for all possible combinations.

Keywords: Urotensin II; Synergism; Response surface

Urotensin II is a potent vasoconstrictor neuropeptide in animals and humans (Bohm and Pernow, 2002). It is now viewed as a mammalian vasoconstrictor that may be a therapeutic target in the management of cardiovascular disease (Douglas and Ohlstein, 2000). This peptide, first isolated from the telofish years ago, has recently become a compound of interest whose inhibition might possibly yield new drug treatments for hypertension and other cardiovascular diseases. The detection of urotensin II prompted the question of whether this peptide might interact with other known endogenous pressor agents such as angiotensin II or norepinephrine. A precise quantitative study of such interactions has not been undertaken, i.e., an examination from complete dose–response data of the individual and combined agents that uses measures of dose equivalence to assess possible departures from simple additivity such as synergism. This kind of analysis, which also forms the basis of the isobolographic method, was carried out from data on isometric tension development that we obtained in isolated rat aorta for urotensin II and angiotensin II, and combination of the two.

All procedures involving the use of animals were approved by the Institutional Animal Care and Use Committee of the Temple University School of Medicine. Adult male Sprague–Dawley rats (300–400 g) were used following a minimum acclimation period of 3 days. Animals were euthanized via CO_2 asphyxiation, and the aorta excised in a manner aimed at preserving the integrity of both the endothelium and adventitia. The integrity of the endothelium was confirmed with carbachol-induced relaxation following pre-contraction with an 80% dose of norepinephrine. Preliminary observations confirmed previously published reports (Kuttan and Sim, 1993) demonstrating tachyphylaxis with the individual agents; therefore, each tissue specimen received only a single dose (or dose combination) in our studies, rather than cumulative addition. It was also noted that contraction following dosing showed a prolonged time to reach maximum (as long as 40 min). The effect examined, isometric tension (above preload level), was normalized to the 120 mM KCl effect. As shown in Fig. 1A, for urotensin II, the maximal effect was estimated to be 72.4% of the KCl maximum and its half-maximal concentration was 6.52±2.13 nM. The corresponding values for angiotensin II were 43.98% of KCl max and 32.09±4.88 nM. Because these individual dose-effect curves reveal a nonconstant potency ratio, the resulting isobole of additivity (not shown) is curvilinear (Grabovsky and Tallarida, 2004) and was used as a guide in determining what

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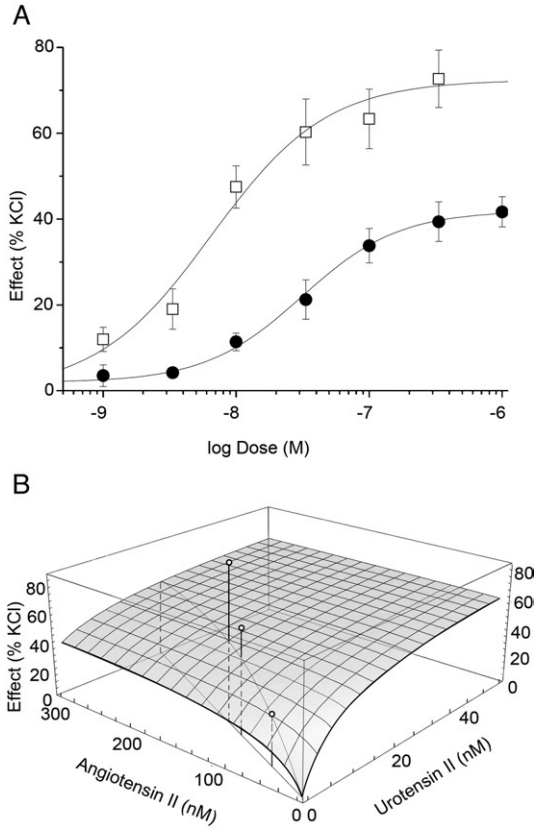

Fig. 1. (A) Dose-effect data (mean ± S.E.M.) and fitted curves of urotensin II (o) and angiotensin II (l) from which the predicted (additive) effect is calculated. Each data point is the mean of 6–8 aortic rings excised from two animals. (B) Effect as a function of drug combination (a, b) is displayed as a surface. Experimentally obtained effect levels (as % KCl) for three combinations with fixed 94:6 ratio are shown and were (total dose): $E_1 = 38.9 \pm 6.8$ (42.5 nM), $E_2 = 77.1 \pm 11.3$ (85 nM) and $E_3 = 83.3 \pm 4.1$ (142 nM). The corresponding additive (surface) effects are: $E'_1 = 34.3 \pm 2.77$, $E'_2 = 44.0 \pm 2.92$ and $E'_3 = 50.2 \pm 2.92$, respectively. The effects for the two largest doses are significantly greater than their corresponding additive effects ($P < 0.05$, t-test for unequal variances, as described in Tallarida, 2000, pp 60–62).

fixed-ratio combination of the agents should be initially tested to assess a possible synergistic interaction.

The individual drug dose-effect data were fitted by nonlinear regression as described by Tallarida (2000) to yield the potency and dose equivalence values that are needed to construct the additive isobole (Grabovsky and Tallarida, 2004; Tallarida, 2000, 2006, 2007). The isobole of additivity, which guided our selection of the combination, is the planar curve of dose pairs that give the constant effect level chosen, in this case half of the maximum of urotensin II. Additivity follows from a computational procedure based on the concept of *dose equivalence*, as in isobolographic analysis. Briefly stated, this procedure uses the doses a of angiotensin II and b of urotensin II by first converting dose a into its b-equivalent (b_{eq}) and then *adding* the b dose. That summed quantity, a function of a and b, yields the additive effect from the urotensin II dose-effect relation. This effect is indicated by the surface height over the domain of a, b combinations and its variance is estimated by the delta method. Experimental results with a fixed-ratio combination (6% urotensin II, 94% angiotensin II) are also shown in the figure and are seen to be positioned above the surface for all total doses >40 nM and these differences are significant ($P < 0.05$, t-test for unequal variances as described by Tallarida, 2000, p. 61.) This finding, indicative of synergism, has no known mechanism, but the finding itself is a first step in the further exploration of this interaction between these two endogenous pressor agents.

Acknowledgments

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