Does biased agonism explain the discrepant pharmacology of ET-1 and S6b?

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We have previously reported discrepancies in the pharmacology of ET-1 and the snake toxin sarafotoxin 6b (S6b) that also binds to and activates ET_A and ET_B receptors (Maguire *et al.,* 1996). Although vasoconstrictor responses to the two agonists were similar in human isolated saphenous vein (that predominantly express ET_A receptors), cross-desensitisation experiments suggested S6b had lower efficacy than ET-1. Additionally, the ET_A selective antagonist BQ123 was a more effective blocker of S6b responses than ET-1, with the Schild-derived affinity of BQ123 dependent on the agonist used. Finally using saturation analysis we discovered that [^{125}I]ET-1 identified a significantly larger population of ET receptors in vein homogenates than [^{125}I]S6b. Based on these data we suggested that ET-1 may bind to an additional population of ET_A receptors that was insensitive to both BQ123 and S6b.

More recently, agonists and antagonists for some G-protein coupled receptors have been shown to demonstrate functional selectivity with respect to activation of signalling pathways. We considered whether this could explain the anomalies in the human ET_A pharmacology of S6b and BQ123. As an initial step we have compared the ability of ET and sarafotoxin peptides to recruit β-arrestin and determined whether this can be antagonised by BQ123 using the DiscoverX Pathhunter Express EDNRA β-arrestin GPCR assay.

CHO-K1 cells expressing human ET_A were incubated with BQ123 (1μM) or vehicle for 60 min at 37ºC. ET peptides/sarafotoxins were added and incubated for a further 90 minutes after which the detection reagent was added and cells incubated for 2 hours at room temperature. The resulting chemiluminescent signal was measured and concentration-response curves to the agonists in the absence and presence of BQ123 expressed as relative light units (RLU).

	Control		**+1μM BQ123**	
	EC_{50} nM	**E_{Max} (RLU)**	**EC_{50} nM**	**E_{Max} (RLU)**
ET-1	0.72	$7.88x10^5$	130	$5.43x10^5$
ET-2	1.65	$6.80x10^5$	124	$3.81x10^5$
ET-3	39.1	$2.49x10^5$	Not determined	
S6b	1.51	$3.33x10^5$	1521	$2.73x10^5$
S6c	No response		Not determined	

Table 1. Potency (EC_{50}) and maximum response (E_{MAX}) of ET and sarafotoxin peptides in the absence (control) and presence of BQ123. Data are derived from 4-parameter logistic curves fitted to the mean of triplicate observations.

These data suggest that for recruitment of β-arrestin the order of agonist potency was ET-1≥ET-2=S6b>ET-3 as expected for an ET_A response and confirmed by the lack of response to the ET_B selective agonist S6c. However, the maximum response to S6b, and ET-3, was approximately half that of ET-1 and ET-2. This may be consistent with S6b activating only a subpopulation of ET_A receptors, as suggested by our saturation binding experiments. Or these data may support the hypothesis that S6b is a biased agonist that couples preferentially to G-protein mediated vasoconstriction but is less likely to cause ET_A receptor desensitisation via β-arrestin recruitment.

Maguire JJ, Kuc RE, Rous BA, Davenport AP (1996). Br J Pharmacol. 118:335-342.