Different pharmacology of ET_A and ET_B receptors revealed in β-arrestin assay

Janet Maguire, Rhoda Kuc, Anthony Davenport. *University of Cambridge, Clinical Pharmacology Unit, Level 6 ACCI, Box 110 Addenbrooke's Hospital Cambridge CB20QQ, UK*

G-Protein coupled receptors may couple promiscuously to multiple G-protein subclasses and may activate intracellular signalling pathways in a G-protein independent manner via the scaffolding proteins β-arrestin 1 and 2. Agonist-specific pathway activation (biased agonism), if identified, may have therapeutic potential. Our aim was to determine the pharmacology of ET_A- and ET_B-mediated β-arrestin recruitment and compare this to the established human pharmacology of these receptors to identify evidence for endothelin (ET) receptor biased signalling and pathway specific blockade by antagonists.

The ability of ET-1, ET-2, ET-3, sarafotoxin 6b (S6b) and sarafotoxin 6c (S6c) to activate ET_A and ET_B-mediated β-arrestin recruitment was investigated using DiscoveRx PathHunter® eXpress β-Arrestin GPCR assays. CHO-K1 cells expressing human ET_A or ET_B were incubated with antagonists or vehicle for 60 min at 37ºC. Increasing concentrations of agonists were added, incubated for 90 minutes, detection reagent was then added and cells incubated for 2 hours at room temperature. The resulting chemiluminescent signal was measured and agonist concentration-response curves expressed as relative light units. Agonist responses were normalised to the maximum response elicited by a control ET-1 curve for each assay. Affinities were obtained for ET_A selective (BQ123, sitaxentan, ambrisentan), ET_B selective (BQ788) and mixed (bosentan) antagonists and compared to affinities obtained in competition experiments in human heart and by Schild analysis in human saphenous vein.

	ET_A			ET_B		
	pD_2	E_{MAX}(% ET-1)	n	pD_2	E_{MAX}(% ET-1)	n
ET-1	9.38±0.09	100±2	11	9.04±0.10	100±2	12
ET-2	8.75±0.05*	87±3*	3	8.55±0.03*	105±2	3
ET-3	7.31±0.05*	31±1*	6	9.44±0.09	83±4*	3
S6b	8.83±0.03*	45±2*	9	9.11±0.08	99±5	7
S6c	No response	-	3	9.10±0.04	96±5	3
BQ3020	No response	-	3	8.94±0.11	82±7	3

Table 1. Potency (pD_2) and maximum response (E_{MAX}, % maximum control ET response) of endothelin and sarafotoxin peptides in ET_A and ET_B expressing cells. Data were derived from 4-parameter logistic curves and are the mean of n=3-12 observations. *Significantly different from ET-1 control (P<0.05, Student's 2-tailed t-test).

For β-arrestin recruitment, the relative potency of ET-1 and ET-3 was as expected for ET_A (ET-1≥ET-2>>ET-3) and ET_B (ET-1=ET-3≥ET-2); for both receptors ET-2 was significantly less potent than ET-1. For ET_A, S6b exhibited comparable potency to ET-2, however, unexpectedly S6b and ET-3 were partial agonists compared to ET-1. As expected S6c was inactive. In the ET_B assay S6b, S6c and BQ3020 showed similar potency to ET-1 and ET-3. Antagonism by selective and mixed antagonists appeared non-competitive, particularly in the ET_A assay. In this assay BQ123 (0.1-1μM) blocked responses to the ET peptides in a comparable concentration-dependent manner with similar data obtained for BQ788 in the ET_B assay. However, BQ123 appeared to be more effective against the sarafotoxins and BQ3020. In competition binding experiments in human heart using [^{125}I]ET-1, bosentan did not distinguish between the two receptor subtypes. However, bosentan (0.3-30μM) produced a greater rightward shift of the ET-1 curve in the ET_A assay than the ET_B assay indicating ET_A selectivity for antagonism of β-arrestin recruitment. The ET_A selectivity of ambrisentan was confirmed and consistent with our human pharmacology for this compound.

In conclusion these data suggest differences in the pharmacology of ET_A and ET_B receptors linked to G-protein- and β-arrestin mediated responses and bosentan appeared to show bias, preferentially blocking ET_A mediated β-arrestin recruitment.