Analysis Of The Influence Of Haplotype On β2-AdrenoceptorInternalisation In Human Stem Cells (HUES7)

The importance of the β-adrenoceptor system in cardiac function is well known and drugs targeting this system, such as certain β-blockers and adrenaline, are prescribed extensively to treat heart failure and cardiac arrest respectively (1). There are a number of single nucleotide polymorphisms within the β2-adrenoceptor (β2-AR) of which Gly16Arg and Glu27Gln have been associated with altered mortality in patients receiving β-blockers after acute coronary syndrome (2). Cardiovascular drug discovery has been limited by the model cell lines available to research the role of β2-AR and to evaluate drugs that target this receptor for disease indications. Often cardiomyoctyes from rats are used in research but within these cells the β2-AR displays altered signalling compared to that in humans (3). To address this we have created human embryonic stem cell (HUES7) lines which stably express one of four SNAP-tagged β2-AR haplo types (varying at sites 16 and 27) and studied the internalisation of these receptors in response to agonist challenge.

HUES7 cell lines (4) stably expressing one of 4 N-terminal SNAP (a self-labelling protein tag) tagged β2-AR haplo types were created (SNAP-β2-AR_R16Q27; R16E27; G16Q27; G16E27). Whole cell radiolig and binding was carried out using ^{3}H-CGP 121771 in serum-free DMEM as previously described (4). For internalisation studies, cells were seeded in 96-well black-walled plates in RPMI-B27 medium. Prior to agonist addition, cells were labelled with SNAP-surface® Alexa Fluor® 488 (New England Biolabs) (0.1 μM) for 30 minutes in medium at 37°C. Cells were then washed and incubated in 100 μl HEPES buffered saline solution in the absence or presence of formoterol, salbutamol and salmeterol (1pM-10 μM) for 1 hr at 37°C. Cells were then fixed, labelled with Hoechst nuclear stain and imaged on the Ultra confocal plate reader (Molecular Devices) the following day. Intracellular receptor was quantified using a granularity analysis algorithm on the MetaXpress software. Data are given as mean±SEM and statistical analysis was performed using two-way ANOVA.

Radioligand binding confirmed typical β2-AR pharmacology in all 4 cell lines with no differences observed in expression level nor the affinity of ^{3}H-CGP121771 and unlabelled ligands (propranolol, CGP 121771, ICI 118551, CGP 20712A). Agonist induced internalisation of the SNAP-tagged β2-AR was confirmed in all 4 haplotypes by confocal imaging. Salmeterol and salbutamol induced internalisation displayedpartial agonism in comparison to formoterol. Agonist potency was conserved across the 4 β2-AR haplotypes with % max salbutamol internalisation significantly greater in R16Q27 compared to the other haplotypes (2-way ANOVA *P<0.05 **P<0.01, Table 1).

Table 1. Agonist potency and maximal internalisation as compared to formoterol.

HUES7 cell line	Formoterol	Salbutamol		Salmeterol	
	EC50	EC50	% max	EC50	% max
SNAP-β2-AR_R16Q27	-7.87±0.06 (7)	-6.29±0.30 (7)	34.4±6.5	-7.43±0.31 (7)	25.8±9.4
SNAP-β2-AR_R16E27	-7.66±0.06(11)	-6.06±0.15 (8)	12.0±4.0**	-7.69±0.38 (7)	10.4±3.4
SNAP-β2-AR_G16Q27	-8.13±0.26 (7)	-5.99±0.19 (6)	13.9±4.3*	-7.32±0.63 (5)	11.4±4.8
SNAP-β2-AR_G16E27	-7.75±0.15 (8)	-5.79±0.06 (7)	12.6±3.7**	-7.40±0.25 (6)	9.8±2.8

In summary, we report the agonist-induced internalisation of SNAP taggedβ2-AR in HUES7 cells expressing different haplo types of the β2-AR.

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(4) Cowan CA *et al.* (2004) N Engl J Med. 350(13): 1353-6;

(5) Baker (2005) Br J Pharmacol .144: 317-322.