A CELL-BASED ASSAY TO MEASURE THE DURATION OF ACTION OF β2 ADRENORECEPTOR AGONISTS AT THE HUMAN RECEPTOR

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Current *in-vitro* methods for determining duration of action of β2 adrenoreceptor agonists typically involve guinea-pig isolated trachea strips in organ bath systems (Coleman *et al* 1989 and 1996) and are often labour intensive and time consuming. We have developed and characterised a cell-based "wash off" assay to assess the duration of action of β2 adrenoreceptor agonists in Chinese Hamster Ovary cells recombinantly expressing the human β2 adrenoreceptor (CHOhβ2).

CHOhβ2 cells were seeded in 96-well Viewplates (Perkin Elmer) at $0.2x10^6$ cells/well in 1% FBS/DMEM-F12 over night at 37°C. Concentration effect curves to β2 adrenoreceptor agonists following a 30-minute incubation at 37°C were constructed by measuring increases in intracellular cAMP levels using a HitHunter cAMP II ™ assay (DiscoveRx). EC_{50} values (concentration of agonist needed to produce half the maximal response to that agonist) for cells stimulated with agonist (unwashed) were compared to EC_{50} values generated in cells exposed to agonists for 30 minutes, washed with PBS and incubated in agonist free media for a further 30 minutes prior to cAMP determination (washed). For ease of comparison, unwashed and washed EC_{50} values for each compound were used to generate a fold rightward shift (RWS) on washing (RWS = EC_{50} washed / EC_{50} unwashed).

The β2 adrenoreceptor agonists caused a concentration related increase in intracellular cAMP with rank order of potency: Formoterol > Salmeterol > Isoprenaline > Salbutamol (see *Table 1*). On washing, the EC_{50} values of the short-acting β2 agonists Salbutamol and Isoprenaline shifted significantly ($p< 0.01$) to the right, whereas the longer-acting β2 agonists Formoterol and Salmeterol produced smaller shifts indicating they were more resistant to washing.

	Unwashed EC_{50} nM	Washed EC_{50} nM	Fold RWS
Salbutamol	25.963 (16.6 – 40.7)	>23051[a](8144 – 65244)	>2003[b](205 – 3847)
Isoprenaline	5.13 (3.14 – 8.39)	1357[a](977 – 1885)	256[b](175 – 373)
Formoterol	0.076 (0.043 – 0.136)	1.941[a](1.25 – 3.01)	27.31[b](12.53 – 51.45)
Salmeterol	1.512 (0.877 – 2.608)	1.923 (1.028 – 3.598)	1.32 (1.013 – 1.598)

Table 1.Comparison of Unwashed and Washed EC_{50} curves. Data are geometric mean and 95% confidence interval values from n=4-9, [a] $p<0.01$ difference between washed and unwashed EC_{50}, [b] $p<0.01$ RWS different from Salmeterol (using ANOVA).

Salbutamol, Isoprenaline, Formoterol and Salmeterol produce statistically distinct washout profiles suggesting that this cell-based assay could be used to rank novel β2 adrenoreceptor agonists on their potency and duration of action prior to any tissue or *in-vivo* determinations. Further experiments are underway to try and determine if the long duration of action is due to “exosite” binding, slow receptor offset or membrane affinity.

Coleman RA *et al*. (1989) *J. Pharmacol Methods*, **21**: 71-86.
Coleman RA *et al.* (1996) *Pulm. Pharmacol*., **9**: 107-117.