**In Vitro Pharmacological Characterisation of the \( \beta_2 \)-adrenoceptor Binding Kinetics of \( [^3H] \)Vilanterol, a Novel Long Acting \( \beta_2 \)-agonist**

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Vilanterol is a novel long acting \( \beta_2 \)-agonist with inherent 24-hour activity in development for inhaled once-daily administration in combination with either an inhaled corticosteroid (for both COPD and asthma) or a long acting muscarinic antagonist (for COPD). It is a potent, highly selective \( \beta_2 \)-adrenoceptor agonist, with significantly greater intrinsic efficacy than salmeterol, significantly greater potency than indacaterol and salbutamol, and significantly greater \( \beta_2 \)-adrenoceptor selectivity than formoterol, indacaterol and salbutamol (Barrett et al. 2010a). In addition, it has been shown in isolated human airways to have a significantly longer duration of action compared to salmeterol, with a significant bronchodilator effect still observed at 22 hours (Barrett et al. 2010b). In this report (funded by GlaxoSmithKline) we describe its affinity and kinetics at the \( \beta_2 \)-adrenoceptor in an *in vitro* radioligand filtration binding assay.

Radioligand binding experiments were performed by filtration with \( [^3H] \)vilanterol and membrane fragments generated from CHO cells expressing human recombinant \( \beta_2 \)-adrenoceptors (non-specific binding defined by 10\( \mu \)M ICI118551) at ambient temperature (20-22°C) in binding buffer (50mM HEPES; 100mM NaCl; 10mM MgCl\( _2 \); pH 7.4). Saturation, association and dissociation binding were investigated (dissociation initiated by 1:20 dilution in binding buffer containing 10\( \mu \)M cold vilanterol). To determine binding parameters at the low and high affinity agonist states of the receptor i.e. G-protein uncoupled and coupled form of the receptor, respectively, experiments were completed +/- 100\( \mu \)M of the GTP analogue 5'-guanylylimidodiphosphate (Gpp(NH)p). All data shown are mean ± SEM, n=4.

Specific binding data from saturation experiments (with ~5pM to 2nM radioligand) were fitted to a one affinity site model +Gpp(NH)p and a two affinity site model -Gpp(NH)p. This analysis resulted in a pK\textsubscript{D} = 9.44 ± 0.07 +Gpp(NH)p and a high affinity site pK\textsubscript{D} = 10.8 ± 0.12 and a low affinity pK\textsubscript{D} = 9.47 ± 0.17 -Gpp(NH)p. Specific binding data from association binding experiments (~0.1 to 2nM radioligand) +/- Gpp(NH)p were both best fitted to a one-phase association model to give a \( k_{on} = 3.84 ± 0.49 \times 10^8 \) M\textsuperscript{-1}min\textsuperscript{-1} respectively, with no significant difference observed between values (p>0.05, Student's t-test). Dissociation binding data (with ~1nM radioligand) were fitted to a one-phase dissociation model +Gpp(NH)p and a two-phase dissociation model -Gpp(NH)p. This analysis resulted in a \( k_{off} = 0.20 ± 0.02 \) min\textsuperscript{-1} (dissociation \( t_{1/2} = 3.5 \) min) +Gpp(NH)p and a fast \( k_{off} = 0.27 ± 0.03 \) min\textsuperscript{-1} (dissociation \( t_{1/2} = 2.6 \) min) and a slow \( k_{off} = 0.015 ± 0.001 \) min\textsuperscript{-1} (dissociation \( t_{1/2} = 46.9 \) min) -Gpp(NH)p.

In conclusion, vilanterol exhibits a high affinity for the \( \beta_2 \)-adrenoceptor coupled with a fast \( k_{on} \). In addition, it demonstrates a fast \( k_{off} \) from the low affinity receptor state and a moderately slow \( k_{off} \) from the high affinity receptor state at ambient temperature. However, even this slower off rate is too fast to explain the functional duration of action of vilanterol observed in isolated human airways, suggesting that receptor kinetics do not explain its long duration of action.
