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Lipid membrane interactions of indacaterol and salmeterol: Do they influence their pharmacological properties?

Dario Lombardi[a,*], Bernard Cuenoud[b,1], Stefanie D. Krämer[a]

[a] Institute of Pharmaceutical Sciences, ETH Zürich, Switzerland
[b] Novartis Institutes for BioMedical Research, Basel, Switzerland

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ABSTRACT

This study compares the lipid membrane interactions of indacaterol, an ultra long acting beta-2 agonist that is given once a day, to salmeterol, a twice a day beta-2 agonist, in order to elucidate the potential mechanisms leading to their different pharmacological properties. Salmeterol but not indacaterol perturbed dimyristoyl-phosphatidylcholine membranes. While the liposome partitioning of the two compounds was similar, independent of the lipid composition, the membrane affinity of indacaterol was two-fold greater than that of salmeterol when rafts, i.e. detergent-insoluble membrane domains, were used as the partition phase. The observed association kinetics with immobilized liposomes at physiological pH were two times faster for indacaterol than for salmeterol. A new model to explain the relationships between the drug/membrane interactions and drug's pharmacological properties considering multiple factors is proposed. The synergy between the higher partitioning of indacaterol into the raft micro domains and the faster membrane permeation of indacaterol could explain the faster onset and longer duration of therapeutic effect of indacaterol. The higher fluidizing effect of salmeterol on membrane fluidity may contribute to its lower intrinsic efficacy compared to indacaterol.

1. Introduction

Inhaled beta-2-adrenoceptor agonists are effective drugs in the management of pulmonary diseases such as asthma and chronic obstructive pulmonary disease (COPD). They induce bronchodilation via direct relaxation of airway smooth muscles, and give rapid relief of symptoms (Barnes, 1977; Waldeck, 2002). Indacaterol, also known as QAB149, is a novel, chirally pure inhaled ultra long beta-2-adrenoceptor agonist, in registration phase for the treatment of COPD. It provides a bronchodilating effect of 24 h after inhalation, combined with a fast onset of action (about 5 min) and an increased efficacy benefit compared with marketed inhaled beta-2-adrenoceptor agonists or the muscarinic receptor antagonist tiotropium (Beeh et al., 2007; Roig et al., 2009). This combination of fast onset, long duration and high efficacy benefit is unique when compared to marketed beta-2-adrenoceptor agonists.

In this study, we investigated whether the beneficial therapeutic profile of indacaterol, in particular the combination of fast onset and long duration, is related to its steady state and kinetic interactions with lipid membranes which provide the environment of the beta-2-adrenoceptor. We, therefore, compared lipid bilayer interactions of indacaterol with those of salmeterol, a beta-2 adrenoreceptor agonist that has been widely used in the past 30 years for the treatment of pulmonary diseases (Ullman and Svedmyr, 1988). Despite similar lipophilicity of the two agonists, salmeterol has a slower onset (around 15 min) and a shorter duration of action (about 12 h) than indacaterol (Lindberg et al., 2007; Palmqvist et al., 1997). Other fast acting agonists, such as salbutamol, have significantly shorter durations of therapeutic action, in agreement with their lower lipophilicity as compared to indacaterol and salmeterol. Beside the differences in time to onset and duration of action, salmeterol and indacaterol differ in their pharmacodynamic characteristics. While indacaterol is a highly efficacious partial agonist at beta-2 adrenoreceptor, salmeterol is a weak partial agonist, which might limit its clinical efficacy (Battram et al., 2006).

Solute-membrane interactions depend on the physicochemical characteristics of the solute and the membrane, respectively. The beta-2-adrenoceptor agonists are characterized by a basic amine

* Corresponding author. Current address: Laboratory of Organic Chemistry, ETH Zurich, Wolfgang-Pauli-Strasse 10, CH-8093 Zurich, Switzerland. Tel.: +41 44 632 4478; fax: +41 44 632 1292.
E-mail address: dario.lombardi@org.chem.ethz.ch (D. Lombardi).
[1] Current address: Merck Serono S.A., Geneva, Switzerland.

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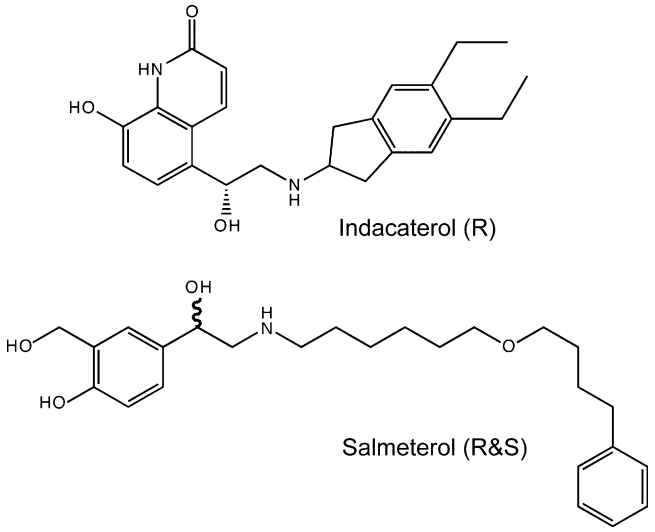

Fig. 1. Chemical structures of indacaterol and salmeterol.

group and an acidic phenol group, as shown in Fig. 1. In solution they can exist in four different ionization forms: the cation (C), two net neutral species (N) including the uncharged species as well as the zwitterionic species, and the anion (A). The acid–base equilibria are defined in terms of the macroscopic constants K_{a1} and K_{a2} that refer to the stoichiometric ionization (Bouchard et al., 2002) where the two net neutral species are treated collectively as being two tautomers of a single form (N). According to the titrated pK_a values of the two compounds, which are pK_a 6.7 and pK_a 8.3 for indacaterol and pK_a 8.8 and pK_a 9.8 for salmeterol, indacaterol is present as a combination of 4 ionization species, predominantly the zwitterion (54.1%) and the neutral one (21.5%) at physiological pH 7.4, while salmeterol is mainly in its cationic form (95.6%) (Cuenoud et al., manuscript in preparation). The fact that zwitterion/neutral species and cations may have different tissue interactions might explain, in part, why indacaterol has a significantly faster onset and duration of action than salmeterol. Indeed bulk hydrophobicity as well as the ionization state have been shown to influence the affinity for liposomes and hence the duration of action of a series of dual dopamine D_2 receptor/beta-2-adrenoceptor agonists (Austin et al., 2003).

We used liposomes of various lipid compositions to investigate a wide range of membrane properties that could influence the interactions with the beta-2-adrenoceptor agonists. Particular attention was given to negatively charged lipids since they are abundant in the lung (Rodgers et al., 2005). We also investigated the effect of cholesterol on the membrane affinities of the drugs, as well as their affinity to extracted membrane rafts since it has been suggested that highly ordered membrane micro-domains are encompassing the beta-2-adrenoceptors and could be of physiological relevance for their function (Ianoul et al., 2005; Pontier et al., 2008; Xiang et al., 2002). Liposomes made of bovine lipid extract surfactant (BLES) were used as a model to investigate the affinity of the agonists to the lung surfactant. Beside the lipid composition, experimental pH and temperature were varied in order to shed light on the influence of the ionization state of drug and lipids and of the physical membrane properties on drug/membrane interactions.

Despite the significant differences in the physicochemical properties of the two drugs, they displayed no major differences in their pH-distribution profiles and membrane interaction kinetics in the liposomal systems. However, the two agonists differed strikingly in their effect on membrane fluidity as determined with the anisotropy probe 1,6-diphenyl-1,3,5-hexatriene (DPH). While indacaterol had no effect, salmeterol significantly increased membrane fluidity at concentrations above 1 μM. Bilayer fluidization by salmeterol may indirectly influence the receptor activation, contributing to the partial agonism properties and the observed slow onset of action of salmeterol. Based on our results, we suggest an adapted model for the relationships between agonist-membrane interactions and their biological effects.

2. Materials and methods

2.1. Chemicals

Salmeterol was obtained from Tocris bioscientific (Ellisville, USA), indacaterol, ^{3}H-indacaterol and ^{3}H-salmeterol were from Novartis (Basel, CH). Propranolol hydrochloride and 1,6-diphenyl-1,3,5-hexatriene (DPH) were purchased from Sigma (Buchs, CH), Zwittergent® 3–14 R from Calbiochem (San Diego, USA). TritonX-100 was purchased from Fluka (Buchs, CH). Complete®, methanol, chloroform, trifluoro–acetic acid and acetonitrile (HPLC grade) for lipid extraction and HPLC investigations were from Merck (Darmstadt, DE). All other chemicals were of analytical grade.

2.2. Lipids

Egg phosphatidylcholine (PhC), spinal cord phospatidylserine (PhS), phosphatidylinositol (PhI) and sphingomyelin (SM), all grade 1, were purchased from Lipid Products (Nutfield, UK). Dipalmitoylphosphoglycerol (DPPG), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylcholine (DOPC), dimyristoylphosphatidylcholine (DMPC), dimyristoylphosphoglycerol (DMPG) and dipalmitoylphosphoethanolammine (DPPE) were from Avanti polar lipids (Alabaster, USA). Cholesterol (Chol) was from Sigma (Buchs, CH). Bovine lipid extract surfactant (BLES) was purchased from BLES Biochemical (London, ON).

2.3. Octanol/buffer partitioning

The distribution profiles in the octanol/buffer system of indacaterol and salmeterol were determined between pH 4 and 13 by the shake flask method (Leo et al., 1971). Propranolol was used as control. Between 5 and 20 μl each of 2 mM methanolic stock solutions of the drugs were added to reaction tubes and the methanol was evaporated. The compounds were re-dissolved in 1 ml water-saturated 1-octanol and 5 ml of standardized universal buffer solutions (SUBS) containing citrate, borate, phosphate and NaCl, adjusted to 0.21 M ionic strength (Pauletti and Wunderli-Allenspach, 1994) at the desired pH. The tubes were shaken for 1 h at room temperature and centrifuged for 10 min at 9000 *g*. Shaking for 3 h revealed the same results, indicating that equilibrium was reached after 1 h. At the end of the experiments no significant pH shifts were observed in the buffer phases. Samples of 200 μl from both phases were diluted with 800 μl methanol and the concentrations were determined by LC/MS/MS with a Waters 2759 HPLC equipped with an Xterra column (C8, 3.5 m, 1 × 50 mm) and an MS Quattro Micro Mass detector. The drugs were eluted with a linear gradient of H_2O/acetonitrile 95%/5% to 100% acetonitrile. The partition coefficients were calculated as the ratios of the concentrations in the respective 1-octanol and aqueous phases.

2.4. Preparation and characterization of liposomes

Liposomes with various lipid compositions (PhC, DMPC, DPPC, DOPC, SM, DMPC/Chol 60/40 mol/mol, SM/Chol 60/40 mol/mol, DOPC/Chol 60/40 mol/mol, PhC/PhS 70/30 mol/mol, DPPC/DPPG 80/20 mol/mol, PhC/DPPG 70/30 mol/mol, DMPC/Chol/