

Solubilisation and binding characteristics of a recombinant β_2 -adrenergic receptor expressed in the membrane of *Escherichia coli* for the multianalyte detection of β -agonists and antagonists residues in food-producing animals

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Abstract

The number of substances with β -agonistic activity, illegally introduced in meat production or in sports doping as anabolic or β -blocking agents is increasing. Analytical methods suited for their multianalyte detection are thus necessary. In this perspective, receptor assays were developed. The research activities undertaken in this study describe the solubilisation of a recombinant human β_2 -adrenergic receptor produced in the inner membrane of genetically modified *Escherichia coli*, using the detergent *n*-dodecyl- β -D-maltoside. Its potential to detect the presence of β -agonists or β -blockers in biological samples was evaluated. The solubilised β_2 -adrenergic receptor retained its binding affinity in a radio-receptor assay based on the competition for the binding to receptors between a ligand (β -agonist or antagonist) and the radioligand [¹²⁵I]iodocyanopindolol. The IC₅₀ values ranged from $5 \pm 1 \times 10^{-8}$ M (clenbuterol) to $8 \pm 2 \times 10^{-6}$ M (isoxsuprine) for the β -agonists tested and from $1.5 \pm 0.2 \times 10^{-10}$ M (carazolol) to $1.2 \pm 0.2 \times 10^{-5}$ M (metoprolol) for the β -blockers tested. It was shown to have a lower limit of detection than a radio-receptor assay using the solubilised β_2 -adrenoceptor expressed in a mammalian cell line. The solubilised recombinant human β_2 -adrenoreceptor expressed in *E. coli* would be a useful tool to develop non radioactive multianalyte screening methods.

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1. Introduction

The use of β -agonists as growth promoters in meat production is banned within the EU (directive 96/22/EC). The consumption of these compounds under the form of residues, especially in liver where the drugs concentrate, but even from meat when large over dosage occurs, can lead to severe intoxication in humans [1–3]. Analytical methods are thus required to monitor the illegal presence of these residues to provide assurance to the consumer on the quality and safety of food and to ensure enforcement

of government tolerances and compliance with export/import requirements. Several analytical methods have been described for the detection of β -agonists residues in urine and animal tissue samples. Confirmatory analysis of β -agonists is based on gas chromatography–mass spectrometry (GC–MS) [4] or liquid chromatography–mass spectrometry LC–MS(–MS) [5], these methods are time-consuming, expensive and unsuited to the screening of large series of samples. The screening methods used are often immunoassays, such as enzyme immunoassay (EIA). Several methods for urine [6–8] and hair [9,10] have been described and various EIA-kits are commercially available. Radio-immunoassays (RIA) have also been developed [11]. A more recent detection technique is immunobiosensor analysis to detect β -agonists in urine or liver [12] and some commercial

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kits using the surface plasmon resonance (SPR) technology are available. All these screening methods have the advantage of offering low limits of detection but they are too selective.

Even using antibodies with broad cross-reactivity, only a limited number of β -agonist residues are detected. With the presence on the black market of new compounds, their detection becomes increasingly problematical as existing analytical methods are not adapted for the detection of new molecules [13]. The advantage of receptor-based assays in residue detection is their capability of detecting a broader spectrum of similar compounds (generic assay), including new structurally related molecules for which no detailed information is known.

The β_2 -adrenergic receptor is a transmembrane protein of G protein-coupled receptors family (GPCRs). Upon receptor activation by an agonistic ligand binding, the receptor induces a conformational change in the associated heterotrimeric G protein. The activation of G protein leads to a signal transduction cascade which mediates cellular responses by modulating specific effector enzymes, such as adenylyl cyclase resulting in an increase of 3'-5'-cyclic adenosine monophosphate (cAMP) [14]. Some authors have reported the effects of agonists and antagonists of β -adrenoceptors in eukaryotic cells quantifying cellular cAMP levels by the use of a luciferase reporter gene assay [15,16]. The goal of these studies was essentially to identify the presence or activity of β -adrenoceptors in particular cell lines or the effect of specific ligands on the receptors instead of developing new β -agonists detection methods.

The β_2 -adrenergic receptor has already been functionally expressed in a variety of different organisms including insect and mammalian cells [17] or *Escherichia coli* [18,19]. Radio-receptor assays (RRA) have already been described for multianalyte detection of β -agonist residues using natural membrane bound β_2 -adrenergic receptors prepared from bovine testis muscles [20], or recombinant β_2 -adrenoreceptors expressed in membranes of a transfected Chinese hamster ovary (CHO) cell line [21] or expressed in human embryonic kidney cells [22]. In the past, we developed a multi-residue screening method for the detection of β_2 -adrenergic residues in urine samples using a radioactive tracer and a genetically modified *E. coli* expressing the human β_2 -adrenergic receptor in their inner membrane [23]. The ultimate objective is to develop a multianalyte detection of β -agonists and β -blockers in biological samples without the use of radioactive ligands, such as dipstick assays. This paper describes the solubilisation of the recombinant human β_2 -adrenergic receptor produced in the inner membrane of genetically modified *E. coli*. The solubilised protein keeps its capacity to bind agonists and antagonists and shows its potential to be used in the development of non radioactive multi-residue screening methods.

2. Experimental

2.1. Chemicals and reagents

Escherichia coli KS303 transformed by pMES plasmid [24] was a gift of A.D. Strosberg (Institut Cochin, Paris, France). The coding regions of the human β_2 -adrenergic receptor were

fused to the promoter of the maltose-binding protein of *E. coli* (MalE) followed by a part of the MalE gene coding for the first 56 amino acids of the MalE protein. The β_2 -adrenergic receptor was produced as a fusion protein with a part of MalE protein to increase the hydrophilicity of the receptor. This hydrophilic partner, as periplasmic protein secreted through the bacterial inner membrane, allows the correct insertion and folding of the receptor in the bacterial membrane.

Luria broth (LB) medium and Luria agar for the maintenance and propagation of *E. coli* were purchased by Gibco-BRL Life Technologies (Gaithersburg, Maryland), as well as ampicillin sodium. Sterilized D-(+)-glucose solution (45%), D-(+)-maltose monohydrate, deoxyribonuclease I, ribonuclease A, phenylmethylsulfonyl fluoride (PMSF), leupeptine, pepstatin A, the detergent *n*-dodecyl- β -D-maltoside, trizma base, potassium phosphate, ethylenediaminetetraacetic acid (EDTA), chloramine T, sodium metabisulfite were supplied by Sigma (St. Louis, Missouri). Lysozyme was purchased from Belovo (Bastogne, Belgium). Iodine resublimed was from UCB (Brussels, Belgium) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes) was purchased by Boehringer (Ingelheim, Germany). Cyanopindolol (CYP) was a gift from Novartis Pharma (Basel, Switzerland). Iodine-125 (1 mCi Na¹²⁵I, 37.0 MBq) was supplied by Amersham Biosciences (Buckinghamshire, UK). The bicinchoninic acid (BCA) protein assay, used for the determination of protein concentration in bacterial preparations, was purchased from Pierce (Rockford, Illinois). All chemicals were of analytical grade.

The β_2 -agonists clenbuterol-HCl, fenoterol-HBr, salbutamol, terbutaline-1/2 H₂SO₄, ritodrine-HCl and β -blockers L-propranolol-HCl, alprenolol-HCl and nadolol were obtained from Sigma (St. Louis, Missouri). The β_2 -agonists carbuterol-HCl, ractopamine-HCl and isoxsuprine-HCl were from BGA (Berlin, Germany), cimaterol was from Boehringer Ingelheim (Mannheim, Germany). The β -blocker carazolol and β_2 -agonist mabuterol were gifts from C.E.R., Laboratory of Hormonology (Marloie, Belgium). The β -blocker metoprolol tartrate was supplied by ICN Biomedicals (Solon, Ohio).

2.2. Equipment

Standard laboratory apparatus was used throughout for the preparation of media and chemical solutions.

A Heraeus minifuge T Centrifuge (Hanau, RFA) was used to recover the bacteria during the β_2 -adrenergic receptor production.

A Centrikon T-2070 ultracentrifuge from Kontron Instruments (Milan, Italy) with Sorvall rotor T-55.38 (Newtown, Connecticut) was used to isolate the bacterial membranes and during the solubilisation steps of the β_2 -adrenergic receptor from the isolated membranes. A MSE Analis sonicator (Suarlée, Belgium) was used for *E. coli* cells disruption. Amicon Centriplus YM-30 from Millipore (Bedford, Massachusetts) was used to concentrate the preparation of solubilised β_2 -adrenergic receptors. A Beckman J2-21 centrifuge with a rotor JA-20 (Palo Alto, California) was used to concentrate the solubilised receptor preparation.

A solvent delivery system Waters 600 (Milford, Massachusetts) and a LC column Symmetry C₁₈ (4.5 × 150 mm, 5 μm) from Waters were used to purify the radioligand [¹²⁵I]-iodocyanopindolol. A 3025 vacuum filtration manifold from Millipore (Bedford, Massachusetts) and glass microfibre filters GF/B from Whatman (Maidstone, UK) were used to filter samples prior analysis by radio-receptor assay. The filters were pre-soaked in 0.5% polyethylenimine (Sigma, St. Louis) to retain, by ionic interactions, the isolated membranes or solubilised proteins on the filters. Radioactivity was measured using a Cobra II Auto-Gamma counter from Packard Instrument Company (Meriden, Connecticut).

2.3. Production of β₂-adrenergic receptors

Recombinant *E. coli* KS303, stored at −70 °C, were grown at 37 °C in LB medium containing 100 μg mL^{−1} ampicillin and 1% D-(+)-glucose (repressing the β₂-adrenergic receptor expression) till an A₆₀₀ value of 1. The bacterial suspension was centrifuged at 3700 × g for 10 min at 4 °C; the bacterial pellet was washed in LB medium free of glucose and centrifuged as described. Expression of the recombinant human β₂-adrenergic receptor was induced in LB medium containing 100 μg mL^{−1} ampicillin and 1% D-(+)-maltose for 5–6 h at 30 °C. The bacterial suspension was centrifuged as described and the bacterial pellet was resuspended in 10 mM Hepes, pH 7.5 and homogenised using a Dounce homogeniser. The final suspension was frozen in liquid nitrogen and stored at −70 °C or used immediately in isolation of cell membranes procedures.

2.3.1. Preparation and fractionation of *E. coli* membranes

E. coli cells were harvested by centrifugation (15 min at 3700 × g and 4 °C) and membrane fractionation was performed by sucrose-density centrifugation using a modification of the method described by Chapot et al. [19]. Bacteria were resuspended in 10 mM Hepes, pH 7.5 containing 20% (m/m) sucrose, 30 μg mL^{−1} deoxyribonuclease I, 30 μg mL^{−1} ribonuclease A, 1 mg mL^{−1} lysozyme, 1 mM PMSF, 5 μg mL^{−1} leupeptine, 7 μg mL^{−1} pepstatine A and sonicated at high power for 2 min, keeping on ice for 30 s between each sonication of 20 s. Unbroken cells were removed by centrifugation at 3700 × g for 15 min at 4 °C. The supernatant was layered on a sucrose gradient consisting of 3 mL 60% (m/v) sucrose, 6 mL 42.5% (m/v) sucrose and 15 mL 25% (m/v) sucrose (in 10 mM Hepes, pH 7.5 containing 5 mM EDTA) and centrifuged at 100,000 × g for 16 h at 4 °C. Two fractions corresponding to the inner and outer membranes of *E. coli* were collected from the gradient, homogenised using a Dounce homogeniser. The final suspension was frozen in liquid nitrogen and stored at −70 °C or assayed for protein concentration by the bicinchoninic acid (BCA) method, similar to the Lowry procedure [25], or used immediately in solubilisation procedures.

2.3.2. Solubilisation of β₂-adrenergic receptors

The membrane suspension was mixed with a 0.5% (m/v) n-dodecyl-β-D-maltoside solution containing 1 mM PMSF, 5 μg mL^{−1} leupeptine and 7 μg mL^{−1} pepstatine A. The sol-

ubilisation of the suspension was performed by four vortexing cycles for 10 s keeping the preparation on ice for 15 min before each agitation. Non solubilised protein was removed by centrifugation at 150,000 × g for 30 min at 4 °C. The resulting supernatant was concentrated by ultrafiltration using an Amicon Centriplus centrifuge filter device. The final solubilised receptor preparation was frozen in liquid nitrogen and stored at −70 °C or assayed for protein concentration by the bicinchoninic acid (BCA) method.

2.4. [¹²⁵I]iodocyanopindolol synthesis

¹²⁵I-CYP was synthesized in our laboratory by a modification of the methods of Engels [26] and Moretti-Rojas [27]. 10 μL of CYP solution (20 μg mL^{−1} in 13 mM HCl) was added to 40 μL of 0.3 M potassium phosphate buffer, pH 7.6 and 10 μL of the mixture were incubated with 1 mCi Na¹²⁵I (10 μL) and 5 mg mL^{−1} chloramine T (10 μL) for 1 min with shaking. Reaction was terminated by adding 10 μL 30 mg mL^{−1} sodium metabisulfite and 0.3 M potassium phosphate buffer, pH 7.6 was added to obtain a total volume of 170 μL. Purification of the radioligand was performed by HPLC. The mobile phase was 10 mM potassium phosphate buffer, pH 7.5 containing 40% methanol (solvent A) or 80% methanol (solvent B). The flow rate was 1 mL min^{−1} and the injection volume was 170 μL. The gradient elution conditions were: from 0 min to 10 min, 100% of solvent A; then from 10 min to 50 min, transition from 100% of solvent A to 100% of solvent B in a linear way. Retention time of iodine-125 was 3 min, unlabelled CYP: 18 min and ¹²⁵I-CYP: 35 min.

2.5. Preparation of standard solutions

Stock solutions of 1 mg mL^{−1} β₂-agonists or β-blockers were prepared by dissolving the compound in methanol and stored at −20 °C. Standard solutions were prepared by dilution of stock solutions with 50 mM trizma base-HCl, pH 8.0. The concentrations ranged from 10^{−4} M to 10^{−10} M for the β₂-agonists and from 10^{−6} M to 10^{−11} M for the β-blockers.

2.6. Binding assays

All binding assays were performed in triplicate in T buffer: 50 mM trizma base-HCl, pH 8.0.

2.6.1. Saturation experiments

150 μL of isolated *E. coli* membranes preparation or membranes solubilise expressing the β₂-adrenergic receptor (20 μg of total proteins) were incubated with 50 μL of increasing concentrations of tracer ¹²⁵I-CYP and 50 μL of T buffer (total tracer binding, Bo) or 50 μL of 10 μM L-propranolol (non-specific binding). After incubation for 30 min at 37 °C, binding reactions were terminated by adding 4 mL of ice cold T buffer and rapid filtration through GF/B glass microfibre filters (pre-soaked in T buffer containing 0.5% polyethylenimine) to separate bound and free fractions. The filters were washed four times with ice

cold T buffer and bound radioactivity was measured with a γ counter.

2.6.2. Inhibition experiments

150 μ L of isolated *E. coli* membranes preparation or membranes solubilisate expressing the β_2 -adrenergic receptor (20 μ g of total proteins) were incubated with 50 μ L of 50 pM tracer 125 I-CYP and 50 μ L of T buffer (Bo) or 50 μ L of 10 μ M L-propranolol (non-specific binding) or 50 μ L of solutions containing increasing concentrations of β -agonists or β -blockers standards. Binding reactions were terminated as described in Section 2.6.1.

2.7. Calculation

Results were calculated by subtracting the non-specific binding counts from the total binding counts at each radioligand concentration (in Section 2.6.1) or at each β -agonists, β -blockers standards concentration (in Section 2.6.2). In receptor assay, the non-specific binding is defined as being the fraction of the total tracer binding to receptors which is not displaced by a high concentration of non radioactive ligand. The β -blocker L-propranolol was used in excess (10 μ M) for the estimation of the non-specific binding in all the experiments.

In Section 2.6.1, results were plotted graphically (specific tracer binding versus tracer concentration). The B_{\max} (concentration of specific binding sites) and K_D values (equilibrium dissociation constant of the tracer) were obtained after Scatchard [28] linear transformation of the data from the saturation curve using Microsoft Excel software.

In Section 2.6.2, the maximum tracer binding expressed in percent was plotted versus log of ligand concentration. The IC_{50} (molar concentration of unlabeled ligand inhibiting binding of radioactive tracer by 50%) was calculated from inhibition curves fitted by linearization using Microsoft Excel where the specific tracer binding expressed in logit function was plotted versus log of ligand concentration. The binding dissociation constant K_i values (concentration of the inhibitor that bind to half of binding sites at equilibrium) were calculated according to the following equation: $K_i = IC_{50} / [2(L - L_0)/L_0 + 1 + (L/K_D)]$ [29] where L_0 and L are the molar concentrations of free 125 I-CYP, respectively, in absence of inhibitor and at the inhibition concentration corresponding to IC_{50} .

3. Results and discussion

In this study, the solubilisation of the human β_2 -adrenergic receptor produced in the inner membrane of genetically modified *E. coli* was successfully performed using the non-ionic detergent *n*-dodecyl- β -D-maltoside. The solubilisation and purification of β_2 -adrenergic receptors in eukaryotic cells were described in many publications but the objective of the authors was generally to obtain large amounts of receptors to study their structural and biochemical properties instead their binding characteristics after the solubilisation procedure. The glycoside digitonin was the non-ionic detergent commonly used to solubilise the β_2 -adrenergic receptor expressed in plasma

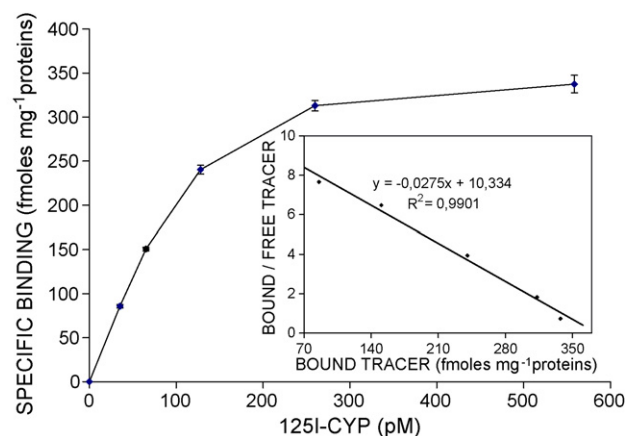


Fig. 1. Specific binding of 125 I-CYP to the β_2 -adrenergic receptor is a saturable process. The Scatchard transformation of the saturation curve given in the inset indicates the presence of a single class of binding sites with a K_D value of 15 ± 4 pM and a B_{\max} value of 378 ± 29 fmol mg⁻¹ of solubilised total proteins. Solubilised β_2 -adrenergic receptor (20 μ g of total solubilised proteins) was incubated at 37 °C with increasing concentrations of tracer 125 I-CYP for 30 min. Results are means of three experiments performed in triplicate.

membranes of eukaryotic cells [30,31]. The alkylglucoside *n*- β -D-dodecyl maltoside, which efficiently solubilise membrane proteins, was used in some publications for the solubilisation of human β_2 -adrenergic receptors from insect cells [32,33]. One method was described for the solubilisation of the human β_2 -adrenergic receptor expressed in the inner membrane of *E. coli* using sucrose dodecanoyl instead *n*- β -D-dodecyl maltoside that, interacting with the MalE part of the fusion protein, prevented the receptor purification on a specific column [34].

The properties of solubilised β_2 -adrenergic receptors were investigated by RRA using the radioactive tracer 125 I-CYP. The specific binding of 125 I-CYP to solubilised receptors was saturable as illustrated in Fig. 1. The Scatchard analysis of the saturation curve given in the inset indicated the presence of a single, homogeneous class of binding sites. The calculated concentration of binding sites B_{\max} (x-intercept) was 378 ± 29 fmol mg⁻¹ of solubilised total proteins. The calculated receptor concentration was 14 ± 1 pmol L⁻¹ of culture medium and 217 ± 18 pmol L⁻¹ of final concentrated preparation. The equilibrium dissociation constant value K_D (the negative reciprocal of the slope) was 15 ± 4 pM. From the results reported in Table 1 was calculated the yield of binding proteins obtained from the different preparations of β_2 -adrenergic receptors: the binding sites recovery from isolated membranes preparation was about 90% in comparison with the B_{\max} obtained with whole bacteria; it was about 57% and 51% from the solubilised receptor preparation in comparison, respectively, with the B_{\max} calculated from isolated membranes and whole bacteria. The receptor concentration in the solubilised preparation was about 2 and 25 times lower in comparison, respectively, with isolated membranes and whole bacteria but the receptor retained its binding affinity for the radioligand after the long steps of the preparation and fractionation of *E. coli* membranes and the solubilisation procedures. Bind-

Table 1

Characteristics of the human β_2 -adrenergic receptor expressed in the inner membrane of *E. coli*

	Whole bacteria	Isolated membranes	Solubilised proteins
B_{\max} values (fmol mg^{-1} total proteins) ^a	739 \pm 54	664 \pm 40	378 \pm 29
Receptor concentration (pmol L^{-1} culture medium)	413 \pm 16	30 \pm 3	14 \pm 1
Receptor concentration (pmol L^{-1} concentrated preparation)	5240 \pm 99	451 \pm 38	217 \pm 18
K_D (pM) ^b	69 \pm 13	16 \pm 2	15 \pm 4

Values are mean \pm s of experiments performed in triplicate ($n=3$)^a Number of binding sites.^b Dissociation constant.

ing characteristics of a solubilised β_2 -adrenoreceptor expressed in membranes of a transfected CHO cell line were previously reported (B_{\max} value of 13 pmol mg^{-1} of membrane proteins and 550 ± 100 fmol mg^{-1} of solubilised proteins; K_D value of 390 pM) [21], as well as a membrane bound β_2 -adrenergic receptor expressed in human embryonic kidney cells (B_{\max} value of 17 pmol mg^{-1} of membrane proteins; K_D value of 281 pM) [22]. Although production level of β_2 -adrenergic receptors reported in *E. coli* was lower than in eukaryotic cells, its affinity for the radioactive ligand (expressed by the K_D value) was higher, indicating a better limit of detection.

The inhibition of ^{125}I -CYP binding to the solubilised receptor was investigated for different β_2 -agonists and β -blockers, with clenbuterol as reference, and the results plotted graphically, as shown respectively in Fig. 2A and B. The IC_{50} values (molar concentration of ligand inhibiting binding of ^{125}I -CYP by 50%) and K_i values (equilibrium dissociation constant of the

inhibitor–receptor complex) for each ligand tested were calculated from sigmoidal inhibition curves linearized as described in Section 2.7 and are listed in Table 2. The solubilised receptor was able to bind a broad spectrum of structurally diverse ligands both β -agonists, as clenbuterol, and β -blockers, as carazolol. From all the molecules tested, the β -blockers carazolol (IC_{50} value of $1.5 \pm 0.2 \times 10^{-10}$ M), L-propranolol (IC_{50} value of $5.0 \pm 1.3 \times 10^{-10}$ M) and alprenolol (IC_{50} value of $1.8 \pm 0.4 \times 10^{-9}$ M) showed the highest binding affinity for the β_2 -adrenergic receptor produced in *E. coli*. From the β -agonists, the aniline-type β -agonists, such as clenbuterol (IC_{50} value of $5 \pm 1 \times 10^{-8}$ M), mabuterol (IC_{50} value of $6 \pm 1 \times 10^{-8}$ M) and cimaterol (IC_{50} value of $1.7 \pm 0.6 \times 10^{-7}$ M) had the highest affinity. The phenolic-type β -agonists, such as ractopamine, salbutamol and fenoterol were recognized by the receptor with a 10 times lower affinity than clenbuterol. The β -agonists terbutaline, ritodrine, isoxsuprine and the β -blocker metoprolol showed the lowest affinity. In comparison with

Table 2

Inhibition of ^{125}I -CYP binding to the solubilised β_2 -adrenergic receptor expressed in *E. coli* (*E. coli*- $\beta_2\text{AR}$) by different ligands, in comparison with the values obtained using a solubilised β_2 -adrenergic receptor expressed in mammalian cells (CHO- $\beta_2\text{AR}$)

	<i>E. coli</i> $\beta_2\text{AR}$		CHO $\beta_2\text{AR}$ ^a	
	Tracer: [^{125}I]iodocyanopindolol		Tracer: [^3H]dihydroalprenolol	
	IC_{50} values ^b (M)	K_i values ^c (M)	IC_{50} values (M)	K_i values (M)
β-Agonists				
Clenbuterol (Reference)	$5.0 \pm 1.0 \times 10^{-8}$	$2.1 \pm 0.4 \times 10^{-8}$	$6.6 \pm 0.7 \times 10^{-7}$	$4.0 \pm 0.4 \times 10^{-7}$
Mabuterol	$6.0 \pm 1.0 \times 10^{-8}$	$2.7 \pm 0.5 \times 10^{-8}$	$4.3 \pm 1.5 \times 10^{-7}$	$2.7 \pm 0.9 \times 10^{-7}$
Cimaterol	$1.7 \pm 0.6 \times 10^{-7}$	$9.0 \pm 3.2 \times 10^{-8}$	$3.7 \pm 1.5 \times 10^{-6}$	$2.3 \pm 0.9 \times 10^{-6}$
Fenoterol	$3.2 \pm 0.2 \times 10^{-7}$	$1.5 \pm 0.1 \times 10^{-7}$	N.A. ^d	N.A.
Ractopamine	$8.0 \pm 1.0 \times 10^{-7}$	$3.8 \pm 0.7 \times 10^{-7}$	$1.1 \pm 0.6 \times 10^{-5}$	$6.9 \pm 3.9 \times 10^{-5}$
Salbutamol	$8.0 \pm 2.0 \times 10^{-7}$	$4.1 \pm 0.9 \times 10^{-7}$	N.A.	N.A.
Carbuterol	$1.4 \pm 0.2 \times 10^{-6}$	$7.0 \pm 1.0 \times 10^{-7}$	N.A.	N.A.
Terbutaline	$5.9 \pm 0.3 \times 10^{-6}$	$2.9 \pm 0.1 \times 10^{-6}$	N.A.	N.A.
Ritodrine	$7.0 \pm 1.0 \times 10^{-6}$	$3.3 \pm 0.4 \times 10^{-6}$	N.A.	N.A.
Isoxsuprine	$8.0 \pm 2.0 \times 10^{-6}$	$3.7 \pm 0.7 \times 10^{-6}$	N.A.	N.A.
β-Blockers				
Carazolol	$1.5 \pm 0.2 \times 10^{-10}$	$3.3 \pm 0.7 \times 10^{-10}$	N.A.	N.A.
L-Propranolol	$5.0 \pm 1.3 \times 10^{-10}$	$1.1 \pm 0.1 \times 10^{-9}$	N.A.	N.A.
Alprenolol	$1.8 \pm 0.4 \times 10^{-9}$	$4.6 \pm 1.3 \times 10^{-9}$	N.A.	N.A.
Nadolol	$3.7 \pm 0.9 \times 10^{-8}$	$5.0 \pm 1.9 \times 10^{-9}$	N.A.	N.A.
Metoprolol	$1.2 \pm 0.2 \times 10^{-5}$	$8.6 \pm 1.7 \times 10^{-6}$	N.A.	N.A.

Values are mean \pm s of experiments performed in triplicate ($n=3$).^a Data from Meenagh et al. [21].^b Molar concentration of ligand inhibiting binding of radioligand by 50%.^c Equilibrium dissociation constant.^d Not available.

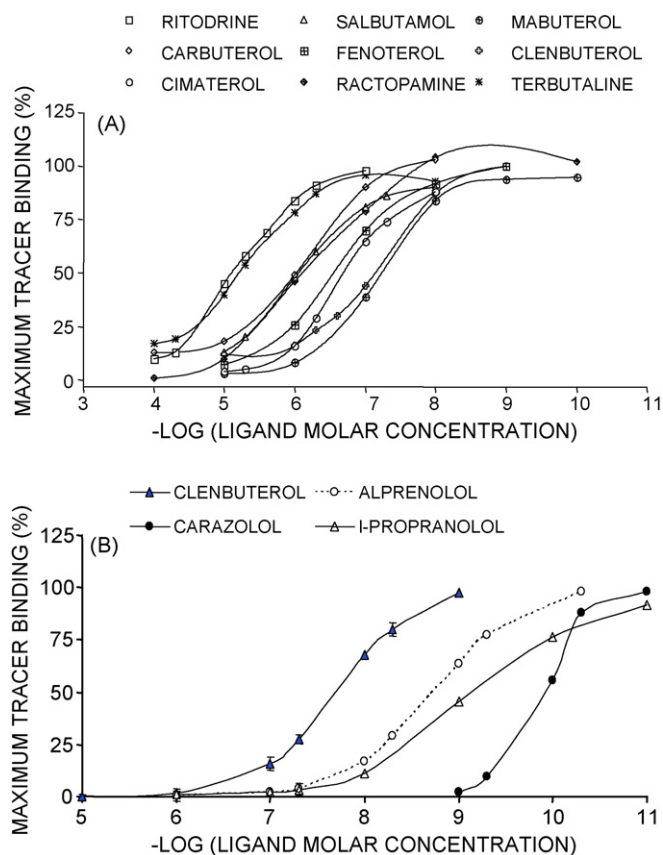


Fig. 2. Inhibition of ¹²⁵I-CYP binding to the β_2 -adrenergic receptor by β -agonists (A) or β -blockers (B) in comparison with the reference β -agonist clenbuterol. Solubilised β_2 -adrenergic receptor (20 μ g of total solubilised proteins) was incubated at 37 °C with 50 pM tracer ¹²⁵I-CYP and increasing concentrations of ligands for 30 min. Results are means of three experiments performed in triplicate.

a radio-receptor assay using the solubilised β_2 -adrenoceptors expressed in membranes of a CHO cell line and the radioligand [³H]dihydroalprenolol [21], the radio-receptor assay using the *E. coli*-receptor should allow a better limit of detection for β -agonist residues, with an affinity for clenbuterol, mabuterol and cimaterol about 10 times and 100 times higher for ractopamine than the CHO-receptor, as shown in Table 2.

4. Conclusions

The results obtained in this study show the ability of the receptor to bind structurally different β -agonists and β -blockers and its potential to detect new compounds with agonistic or antagonistic activity. Furthermore, manipulations of recombinant proteins produced in *E. coli* can be performed, under reproducibility conditions, more quickly and at a lower cost than in eukaryotic cells. In conclusion, the solubilised recombinant β_2 -adrenergic receptor would be a useful tool to develop non radioactive multianalyte screening methods in analysis of residues in foods of animal origin or in living animals, as well as for the anti-doping control of the human athletes or racehorses.

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