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The effect of stereochemistry on the thermodynamic characteristics of the binding of fenoterol stereoisomers to the β2-adrenoceptor

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Abstract

The binding thermodynamics of the stereoisomers of fenoterol, (R,R')-, (S,S')- , (R,S')-, and (S,R')-fenoterol, to the β_2-adrenergic receptor (β_2-AR) have been determined. The experiments utilized membranes obtained from HEK cells stably transfected with cDNA encoding human β_2-AR. Competitive displacement studies using [^{3}H]CGP-12177 as the marker ligand were conducted at 4°, 15°, 25°, 30° and 37°C, the binding affinities calculated and the standard enthalpic ($\Delta H°$) and standard entropic ($\Delta S°$) contribution to the standard free energy change ($\Delta G°$) associated with the binding process determined through the construction of van't Hoff plots. The results indicate that the binding of (S,S')- and (S,R')-fenoterol were predominately enthalpy-driven processes while the binding of (R,R')- and (R,S')-fenoterol were entropy-driven. All of the fenoterol stereoisomers are full agonists of the β_2-AR, and, therefore, the results of this study are inconsistent with the previously described "thermodynamic agonist-antagonist discrimination", in which the binding of an agonist to the β-AR is entropy-driven and the binding of an antagonist is enthalpy driven. In addition, the data demonstrate that the chirality of the carbon atom containing the β-hydroxyl group of the fenoterol molecule (the β-OH carbon) is a key factor in the determination of whether the binding process will be enthalpy-driven or entropy-driven. When the configuration at the β-OH carbon is S the binding process is enthalpy-driven while the R configuration produces an entropy-driven process.

Keywords

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1. Introduction

The thermodynamics of the binding of agonists and antagonists to β-adrenergic receptors (β-ARs) have been described as fundamentally different processes in which the binding of an agonist is enthalpy-driven while the binding of an antagonist is entropy-driven [1–3]. This observation has been generalized as the principle of "thermodynamic agonist-antagonist discrimination", which has been defined as a relationship that when the binding of an agonist is entropy-driven, the binding of an antagonist is enthalpy driven, or vice versa. [4]. This principle has been recently reviewed and appears to hold for the β-AR, adenosine A_1 and A_{2A}, A_{2B} and A_3, cannabinoid CB_1 and CB_2, histamine H_3, glycine, $GABA_A$, serotonin 5-HT_3, nicotinic acetylcholine and purinergic $P2X_3$ receptors [4,5]. However, "thermodynamic agonist-antagonist discrimination" does not appear to apply to cholecystokinin CCK2, dopamine D_2, histamine H_1, δ- and μ-opiod, purinergic $P2X_1$ and serotonin 5-HT_{1A} receptors [4,5].

To our knowledge, the data used to establish "thermodynamic agonist-antagonist discrimination" at the β-AR were obtained using racemic mixtures or only one enantiomer of a chiral compound. One of these compounds was the agonist fenoterol. Fenoterol, is an asymmetric molecule that contains two chiral centers and, therefore, exists as 4 stereoisomers, (R,R')-, (S,S')-, (R,S')-, (S,R')-fenoterol, Fig. 1, where the configuration at the carbon containing the β-hydroxyl moiety, β-hydroxy carbon, is denoted as R or S and the configuration at the chiral center on the aminoalkyl portion of the molecule is denoted as R' or S'. The compound identified as "fenoterol" in the initial study by Wieland, et al. [3] was the racemic (50:50) mixture of (R,R')- and (S,S')-fenoterol, *rac*-fenoterol.

We have recently synthesized all of the stereoisomers of fenoterol and demonstrated that the chirality at the two chiral centers affected the magnitude of the interactions with the $β_2$-AR. In these studies, the $β_2$-AR binding affinities (K_i) and activities (EC_{50}) were determined using a stably expressed cell line [6,7,]. The calculated K_i values differed for each stereoisomer and ranged from 7158 nM (S,S')-fenoterol to 164 nM (R,R')-fenoterol (25°C), see Table 1. The data also demonstrated that all of the fenoterol stereoisomers were full agonists, defined as ≥ 100% induced stimulation of cAMP accumulation relative to isoproterenol, with EC_{50cAMP} values of 0.3 nM (R,R'), 4.70 nM (R,S'), 8.50 nM (S,R') and 580 nM (S,S') [7] and Supplemental Data, Figs. S1-4. The activity of the fenoterol stereoisomers also differed in a rat cardiomyocyte contractility model with $EC_{50cardio}$ values of 73 nM (R,R') [8], 575 nM (R,S') [7], 2,340 nM (S,R') [8], and 55,000 nM (S,S') [unpublished data]. In addition, we have also demonstrated that the stereochemistry of the fenoterol molecule determines the $β_2$-AR coupling preference for G-proteins in cardiomyocyes, as (R,R')-fenoterol preferentially activates G_s signaling while (S,R')-fenoterol activates both G_s and G_i proteins [8].

The objective of the current study was to further explore the role that stereochemistry plays in the interactions of fenoterol with the $β_2$-AR through the determination of the binding thermodynamics of the four fenoterol stereoisomers. The results indicate that the binding of (S,S')-fenoterol and (S,R')-fenoterol were enthalpy-driven, while the binding of (R,R')-fenoterol and (R,S')-fenoterol was entropy-driven. Thus, the binding of these compounds to the $β_2$-AR does not conform to the previously described "thermodynamic agonist-antagonist discrimination" and instead is similar to the processes described for the cholecystokinin CCK2, dopamine D_2, histamine H_1, δ- and μ-opiod, purinergic $P2X_1$ and serotonin 5-HT_{1A} receptors, in which agonists bind to the receptors with both enthalpy-driven and entropy-driven mechanisms. In addition, the data demonstrate that the chirality of the β-OH carbon is a key factor in the determination of whether the binding process will be enthalpy-driven or entropy-driven, with the S configuration resulting in an enthalpy-driven and the R configuration producing an entropy-driven process. To our knowledge this is the first report in which the

binding thermodynamics of enantiomers differ not only quantitatively but also qualitatively, i.e. the binding of one enantiomer is enthalpy driven ($\Delta H° < 0$) while the binding of the other is purely entropy-driven ($\Delta H° \geq 0$).

2. Materials and Methods

2.1. Materials

(R,R')-Fenoterol, (R,S')-fenoterol, (S,R')-fenoterol and (S,S')-fenoterol were synthesized as previously described [6]. [5,7-^{3}H]-(-)-CGP-12177 was purchased from PerkinElmer (Shelton, CT), DMEM was purchased from Lonza Walkersville, Inc. (Walkersville, MD), Fetal Bovine Serum was purchased from Atlas Biologicals (Fort Collins, CO), Penicillin-Streptomycin andGeneticin (G418) were purchased from Invitrogen (Carlsbad, CA), Sodium Chloride and Calcium Chloride were purchased from Mallinckrodt (Phillipsburg NJ) and (±)-Propranolol, (-)-Isoproterenol, Tris-HCL, Trizma Base, Potassium Chloride, Magnesium Chloride, D-(+)-Glucose were purchased from Sigma-Aldrich (St. Louis, MO).

2.2 Membrane binding studies

The binding affinities, expressed as K_i values, were determined as previously described [6,7]. In brief, HEK cells stably transfected with cDNA encoding human β2-AR (provided by Dr. Brian Kobilka, Stanford Medical Center, Palo Alto, CA) were grown in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) and 0.05% penicillin-streptomycin with 400 μg/ml G418. The cells were scraped from the 150×25 mm plates and centrifuged at $500 \times g$ for 5 min. The pellet was washed twice by homogenization in 50 mM Tris-HCl, pH 7.7 and centrifugation at $27,000 \times g$ for 10 min. The pellet was resuspended in 25 mM Tris-HCl, containing 120 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl2, 0.8 mM MgCl2, and 5 mM glucose, pH 7.4. The binding assays contained 0.3 nM [^{3}H]CGP-12177 in a volume of 1.0 ml. Nonspecific binding was determined using 1 μM propranolol. Binding measurements were conducted in triplicate at 4°, 15°, 25°, 30° and 37°C. To reach equilibrium, the incubation lasted 1.5 h at 4°, and 1 h for the experiments conducted at 15°, 25°, 30°, and 37°C. The buffer was adjusted to maintain pH 7.4 at each temperature. As previously described (6,7), the data was used to construct binding curves, which were used to determine IC_{50} values and Hill coefficients using Prism software (GraphPad Software, Inc., San Diego, CA) running on a personal computer and K_i values were then determined using the Cheng-Prusoff transformation.

2.3 Calculations

The enthalpic ($\Delta H°$) and entropic ($\Delta S°$) contribution to the standard free energy change of binding ($\Delta G°$) were determined using the following equation:

$$\ln K_i = \frac{\Delta S°}{R} - \frac{\Delta H°}{R} - \frac{1}{T}$$

(1)

where K_i is equilibrium binding constant, R is the gas constant (8.314 J mol^{-1}K^{-1}) and T is the temperature of experiment in kelvin. The van't Hoff plots were constructed by plotting ln K_A versus 1/T, $\left(K_A = \frac{1}{K_i}\right)$ and the slope and the intercept were used to estimate both $\Delta H°$ and $\Delta S°$ values. Since the binding affinity of (R,R')-fenoterol did not depend upon temperature, the correlation examined using eqn. (1) was not statistically significant. In this case, the slope was assumed to be zero and the intercept was calculated as the average of $\ln K_A$ values determined over the temperature range.

3. Results

The effect of a decrease in temperature, from 37°C to 4°C, on the K_i values of the fenoterol stereoisomers is reported in Table 1. The agonist (R)-isoproterenol and the antagonist *rac*-propranolol were utilized as a prototypical agonist and antagonist as the thermondynamics of the β_2-AR binding of these compounds have been previously examined [1–4]. The results obtained for these two compounds are also presented in Table 1. The binding affinity, K_d value, of the marker ligand, [3H]-CGP-12177, was also determined over the range of experimental temperatures and the results are presented in the Supplemental Data, Table S1. At each temperature, the K_d value of the marker at that temperature was used in the calculation of the K_i values of the displacers.

All of the binding curves constructed in this study were monophasic and indicative of an interaction with one homogenous class of binding sites, although steeper slopes were observed with (S,S')-fenoterol and (S,R')-fenoterol. This is illustrated by the binding curves for (R,R')-fenoterol and (S,S')-fenoterol at 4°C and 37°C, Fig. 2. Hill coefficients were determined for all of the binding experiments, Table 1. The calculated coefficients for (R,R')-fenoterol, (R,S')-fenoterol, (R)-isoproterenol and *rac*-propranolol were close to 1.0, while the Hill coefficients for (S,S')-fenoterol and (S,R')-fenoterol were significantly greater than 1.0. While the meaning of these differences has not been determined, they appear to be associated with the stereochemistry at the beta-hydroxy carbon and suggest that the chirality at this carbon affects the mode of binding.

In this study, the reduction in temperature produced a significant reduction in the K_i values for (S,S')-fenoterol and (S,R')-fenoterol with an affinity difference between the lowest and highest temperature, ΔK_i of −11683 nM and −2740 nM, respectively. The significance of the difference was determined using a two-tailed t-test for independence of two means and the calculated p values were 0.017 ((S,S')-fenoterol) and 0.013 ((S,R')-fenoterol). There was a significant decrease in the K_i values for (R)-isoproterenol with decreasing temperature, ΔK_i −210 nM (p = 0.003), which is consistent with the previously reported data [1–3]. The results are consistent with the description of the effect of temperature on the binding affinities of β_2-AR agonists in which there are large increases in binding affinities with decreasing temperatures [1–3].

The opposite effect was observed with (R,S')-fenoterol, where the K_i values increased with decreasing temperature and the ΔK_i value was +741 nM (p = 0.015). For (R,R')-fenoterol the ΔK_i was +14 nM, but this difference was not statistically significant, p = 0.5740, as the ΔK_i was lower than the average standard deviation of K_i determination which was 23 nM. In case of *rac*-propranolol the ΔK_i was was also small (−0.09 nM) but this time the difference was statistically significant, p = 0.014. The data indicate that temperature had little effect on the affinity of (R,R')-fenoterol or *rac*-propranolol. The observed results for propranolol are consistent with the general observation that changes in temperature have little effect on the binding affinities of β_2-AR antagonists [1,2], however inconsistent for full β_2-AR agonists such as (R,R')-fenoterol and (R,S')-fenoterol.

The data in Table 1 were used to construct van't Hoff plots, Fig.2, and to calculate the entropic ($\Delta S°$) and enthalpic ($\Delta°$) components of the binding process, Table 2. The van't Hoff plots for (S,S')-fenoterol, (S,R')-fenoterol and (R)-isoproterenol were linear with positive slopes, and the calculated $\Delta H°$ values were highly negative and the $-T\Delta S°$ values ranged from −19.54 kJ mol^{-1} ((R)-isoproterenol) to close to zero for (S,S')-fenoterol and (S,R')-fenoterol. The results indicate that the binding of these compounds were primarily enthalpy-driven. In contrast, the van't Hoff plot for (R,S')-fenoterol was linear with a negative slope, the calculated $\Delta H°$ value was close to zero (7.1 kJ mol^{-1}) and the $-T\Delta S°$ value was −40.0 kJ mol^{-1}. In case of (R,R')-fenoterol, where van't Hoff correlation was not statistically significant, the slope (and $\Delta H°$)

was assumed to be zero and the intercept was calculated by averaging the $\ln K_i$ values over the temperatures. The intercept was then used to calculate $\Delta S°$ and the $-T\Delta°$ values for (R,R')-fenoterol, -38.8 kJ mol^{-1}. The results for (R,S')-fenoterol, (R,R')-fenoterol and *rac*-propranolol indicate that the binding processes for these compounds were primarily entropy-driven. The thermodynamic parameters, $\Delta H°$ and $T\Delta S°$, for each compound were plotted following the approach reviewed by Borea, et al [4], Figure 3. The resulting figure presents a visual representation of the thermodynamic data and a simplified classification of ligand-receptor interactions according to their presence in quadrants labeled as enthalpy driven, enthalpy-entropy driven or entropy-driven processes. The $T\Delta S°$ and $\Delta H°$ plot using the data from this study indicated that the binding of (S,S')-fenoterol to the β_2-AR was within the quadrant associated with a purely enthalpy controlled process, the binding of (S,R')-fenoterol, (R)-isoproterenol and *rac*-propranolol were located within the quadrant associated with an enthalpy-entropy driven process and the binding of (R,S')-fenoterol and (R,R')-fenoterol and were within the quadrant associated with a purely entropy controlled process, Fig. 3. The results obtained with (R)-isoproterenol and *rac*-propranolol were consistent with previously reported plots [4]. When the previously reported $\Delta H°$ and $T\Delta S°$ parameters for *rac*-fenoterol [1] were plotted, the compound was placed within the quadrant associated with an enthalpy-entropy driven process and lay between (S,S')- and (R,R')-fenoterol, Fig. 3. This is consistent with the data from this study as results for the *rac*-fenoterol would reflect the contribution of both enantiomers. In addition, it is interesting to note that the K_d values associated with the binding of [^{3}H]-($-$)-CGP-12177 were temperature dependent and decreased with increasing temperature, Supplemental Data, Table S1. The data was used to construct a van't Hoff plot which was linear ($R^2 = 0.972$) and which allowed the calculation of $\Delta H = +20.9$ (± 2.0) kJ/mol and $-T\Delta S = -76.4$ (± 2.0) kJ/mol. The results locate the compound within the quadrant associated with an enthalpy controlled process as would be expected for an antagonist.

4. Discussion

All of the fenoterol stereoisomers used in this study are full agonists of the β_2-AR, and, therefore, the results of this study are inconsistent with the hypothesis that agonist binding to the β-AR [1] and β_2-AR [2] is an enthalpy-driven process. Instead, the data indicate that agonist binding to the β_2-AR is similar to the processes described for the cholecystokinin CCK2, dopamine D_2, histamine H_1, δ- and μ-opiod, purinergic $P2X_1$ and serotonin $5-HT_{1A}$ receptors, in which agonists bind to the receptors with both enthalpy-driven and entropy-driven mechanisms [4,9].

Since the physicochemical properties of enantiomers are essentially equivalent, the thermodynamic differences between the two enantiomeric pairs, i.e. (R,R')- and (S,S')-fenoterol and (R,S')- and (S,R')-fenoterol, reflect the specific binding interactions with the β_2-AR. The designation of the binding process as enthalpy-driven or entropy-driven is the sum of the enthalpic ($\Delta H°$) and entropic ($\Delta S°$) contributions of these interactions; in which $\Delta H°$ is associated with hydrogen bond formation, polar interactions and van der Waals interactions and $\Delta S°$ reflects hydrophobic interactions produced by an increase in solvent entropy arising from the conformational changes in the receptor involving hydrophobic groups and the release of water upon binding [3,5,10].

Based on the data from this study, the chirality of the carbon atom containing the β-hydroxyl group of the fenoterol molecule (the β-OH carbon) is a key factor in the determination of whether the binding process will be enthalpy-driven or entropy-driven. When the configuration at the β-OH carbon is S, the binding process is enthalpy-driven while the R configuration produces an entropy-driven process. While the effect of the stereochemistry at the β-OH carbon on the thermodynamics of agonist binding to the β_2-AR has not been previously examined, the chirality at the β-OH carbon has been shown to affect the binding kinetics, affinities and agonist

activities, c.f. [8,11–14]. These differences have been associated with enantioselective binding interactions between the β-OH moiety and the Asn-293 residue in TM6 of the protein, in which the R-configuration produced the more favorable complex [11]. Another description of the effect of the chirality of the β-OH carbon on agonist activities and binding kinetics was based upon the assumption that the β_2-AR exists in an inactive (R) state and one or more ligand-specific active conformations ($R*^n$) and that stereochemistry plays a role in the conformational response to agonist binding [12,13]. A recent computational study of the docking of enantiomeric β_2-AR agonists and antagonists with a human β_2-AR model has raised the possibility that (R)- and (S)-enantiomers interact with different residues on the receptor [14].

We have previously described the binding of fenoterol and fenoterol derivatives as involving interactions with two binding areas of the β_2-AR [6,7]. In this model, the "catechol" portion of the molecule interacts with the area created by the transmembrane (TM) helices defined by TM3, TM5 and TM6 ("catecholamine site"). The binding of agonists and antagonists within this area has been extensively studied, c.f. [11–13] and attributed to a combination of ionic, hydrogen bonding and π–π or π-hydrogen bond interactions. It is reasonable to assume that this process would be predominately described as an enthalpy-driven process. Fenoterol also contains a 4-hydroxyphenyl moiety on the aminoalkyl portion of the molecule, Fig. 1, and additional binding interactions have been suggested between the aromatic substituent and a second binding area defined by TM3, TM6 and TM7 ("hydrophobic site"). These interactions include hydrophobic and π–π or π-hydrogen bond interactions and hydrogen bond formation involving the 4-hydroxy moiety [6,7]. It is reasonable to assume that binding within this site would be predominantly described as an entropy-driven process.

The results of this study are consistent with a binding process that includes interactions with both of the identified binding areas and in which the relative importance of these interactions is indicated by the thermodynamic profile. Thus, for (R,R') and (R,S')-fenoterol, the interactions of the aminoalkyl portion of the molecule within the "aromatic site" are of greater importance in the formation and stabilization of the fenoterol-β_2-AR complex than the interactions with the binding area defined as the "catecholamine site". The inverse situation is suggested by the data obtained with (S,S')- and (S,R')-fenoterol.

The data does not permit a definitive determination of whether the change from an enthalpy-driven process to an entropy-driven process is a result of enantioselective interactions with different residues in the receptor or the induction of enantiomer-specific conformations. In the current study, [³H]-CGP-12117 was used as the marker ligand. CGP-12117 is a high-affinity neutral antagonist which acts at the "catecholamine site," although at high concentrations the compound binds to at least one additional site on the β1-AR and acts as an agonist [15]. Thus, the enantioselective differences in the K_i values reflect the relative affinity of the fenoterol stereoisomers for an inactive receptor state. The determination of the effect of the stereochemistry at the β-hydroxy carbon on the binding to an active state of the receptor would provide additional insight into this issue. We have recently reported the synthesis of [³H]-labeled (R,R')-4-methoxyfenoterol [16], an analogue of fenoterol with full β_2-AR agonist activity [7,8]. Displacement studies using this compound as the marker ligand are currently in progress and the results will be reported elsewhere.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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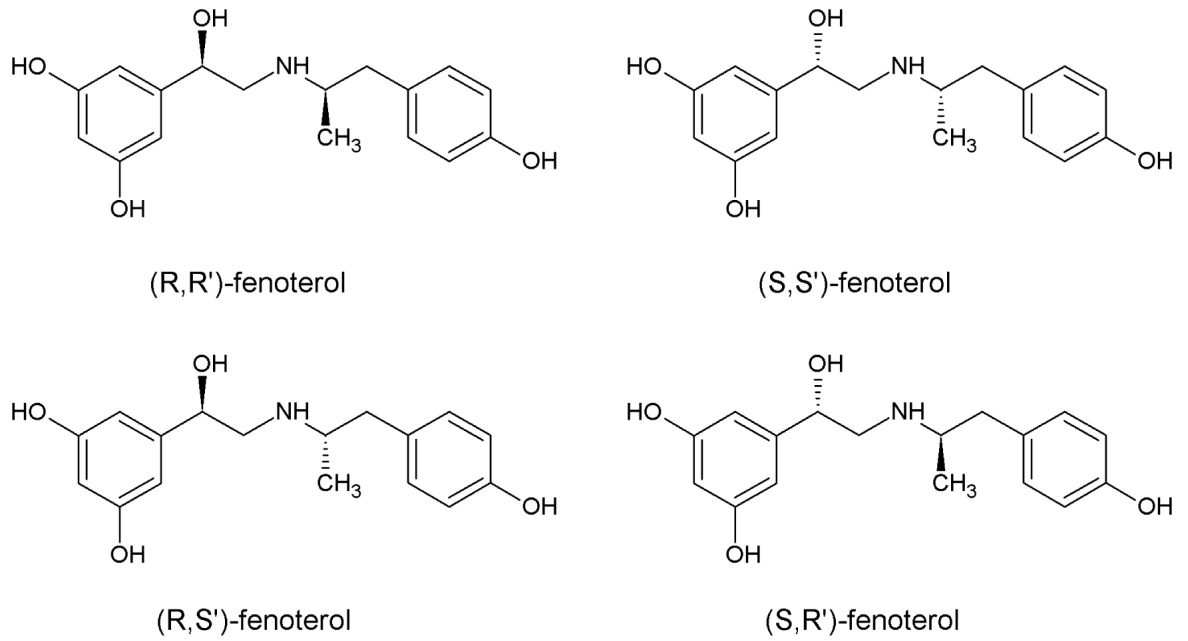
(R,R')-fenoterol

(S,S')-fenoterol

(R,S')-fenoterol

(S,R')-fenoterol

Figure 1.
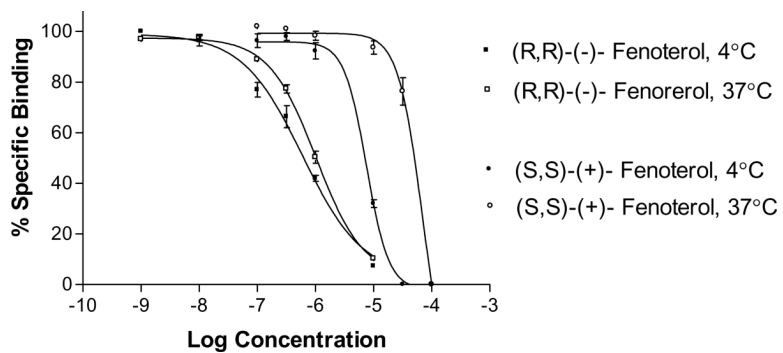Structures of the compounds used in this study.

Figure 2.
The displacement of 0.3 nM [^{3}H]CGP-12177 by (R,R')-fenoterol and (S,S')-fenoterol from membranes obtained from HEK293 cells stably transfected with cDNA encoding human β2-AR obtained at 4°C and 37°C. See text for experimental details.
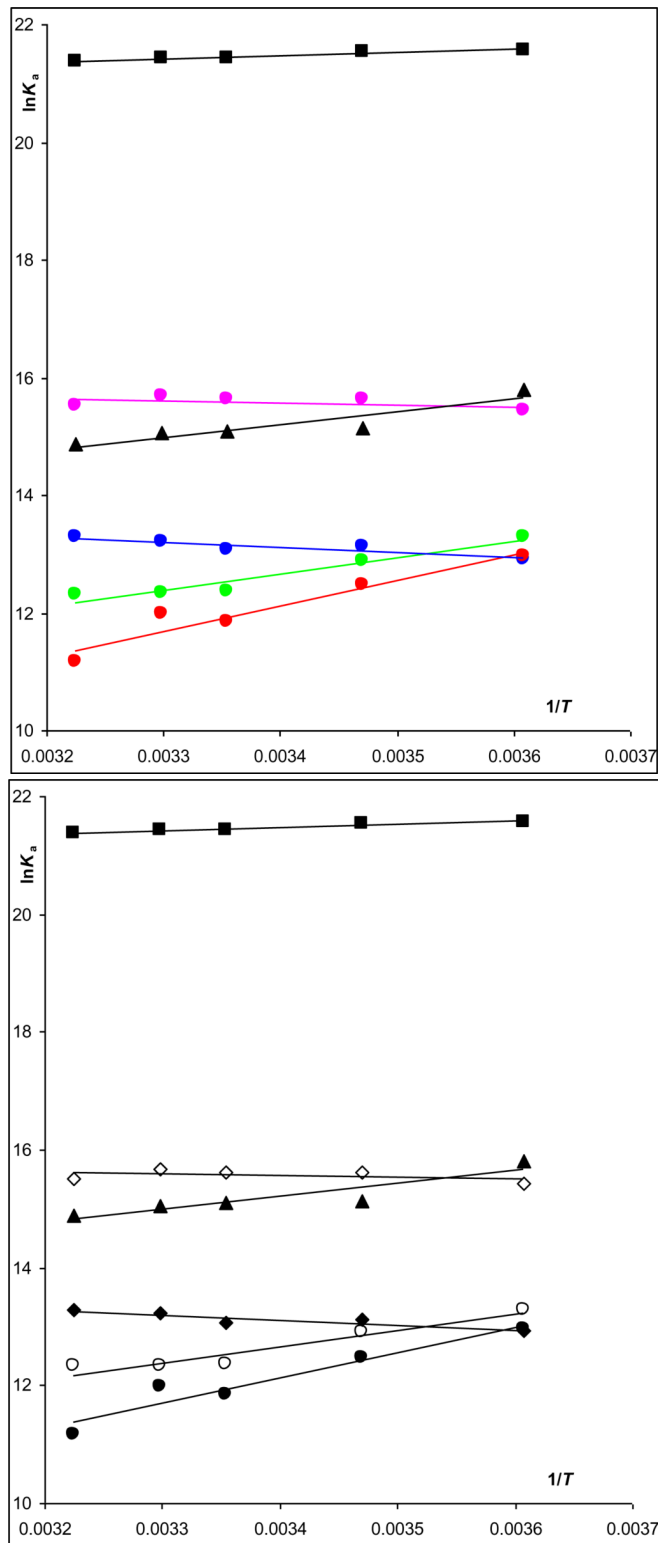
Figure 3.
(Online version) Van't Hoff plots for (S,S')-fenoterol (●), (S,R')-fenoterol (●), (R,S')-fenoterol (●), (R,R')-fenoterol (●), *rac*-propranolol (■) , (R)-isoproterenol (▲).

(Black and White print version) Van't Hoff plots for (S,S')-fenoterol (●), (S,R')-fenoterol (○), (R,S')-fenoterol (◆), (R,R')-fenoterol (◊), *rac*-propranolol (■) , (R)-isoproterenol (▲).
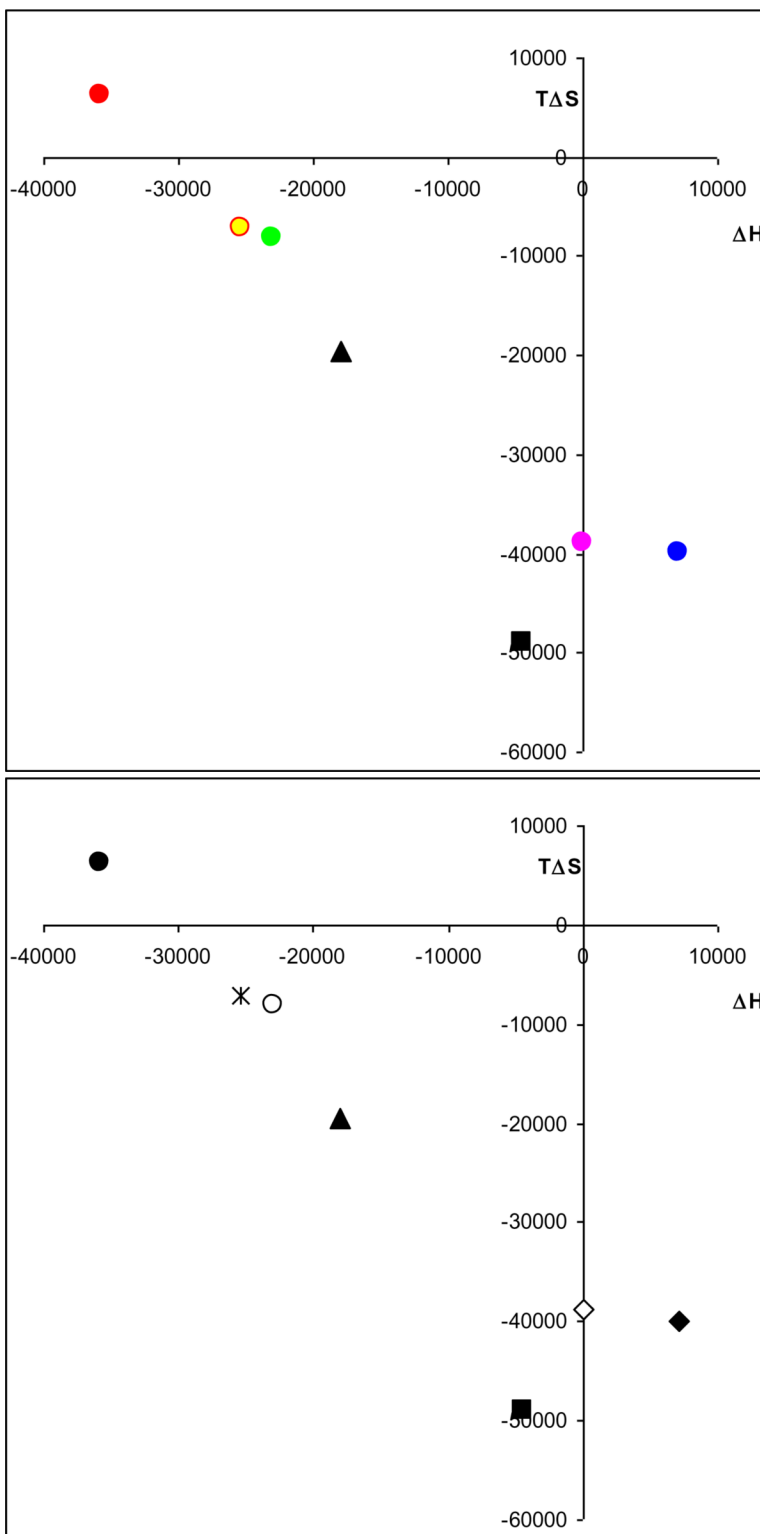
Figure 4.
(Online version) Enthalpy/entropy relation plot for (S,S')-fenoterol (●), (S,R')-fenoterol (●), (R,S')-fenoterol (●), (R,R')-fenoterol (●), *rac*-propranolol (■), (R)-isoproterenol (▲). The plot indicates also the location determined for *rac*-fenoterol (○) by Weiland et.al [1].

(Black and White print version) Enthalpy/entropy relation plot for (S,S')-fenoterol (●), (S,R')-fenoterol (○), (R,S')-fenoterol (◆), (R,R')-fenoterol (◊), *rac*-propranolol (▉) , (R)-isoproterenol (▲). The plot indicates also the location determined for *rac*-fenoterol (☼) by Weiland et.al [1].

Table 1

The K_i values (nM ± SD) and Hill's coefficient (n ± SD) determined for the four stereoisomers of fenoterol, (R)-isoproterenol and rac-propranolol at five different temperatures; n = 3 for each determination unless otherwise indicated. ΔK_i is a change in K_i values between the lowest and the highest temperature (i.e., K_i (4°C)-K_i (37°C)) and the significance, p, was calculated using a two-tailed t-test for independence of two means: K_i (4°C) and K_i (37°C); a $p \leq 0.05$ is assumed a significant difference in ΔK_i.

Temp [°C]	(S,S')-(+)-fenoterol	(S,R')-(+)-fenoterol	(R,S')-(−)-fenoterol	(R,R')-(−)-fenoterol	(R)-isoproterenol	rac-propranolol*
4°C						
K_i	2325 ± 124	1700 ± 372	2450 ± 246	198 ± 33	137 ± 19	0.43 ± 0.09
n	1.69 ± 0.20	1.62 ± 0.38	1.05 ± 0.18	0.87 ± 0.10	0.83 ± 0.03	1.12 ± 0.16
15°C						
K_i	3838 ± 295	2507 ± 232	2001 ± 200	164 ± 13	268 ± 38	0.44 ± 0.08
n	2.06 ± 0.14	1.95 ± 0.04	1.08 ± 0.19	0.92 ± 0.15	0.98 ± 0.13	1.14 ± 0.11
25°C						
K_i	7158 ± 663	4293 ± 374	2087 ± 114	164 ± 31	285 ± 23	0.46 ± 0.06
n	2.04 ± 0.04	1.63 ± 0.24	1.12 ± 0.11	0.88 ± 0.05	0.99 ± 0.08	1.10 ± 0.08
30°C						
K_i	6237 ± 252	4430 ± 268	1809 ± 132	156 ± 17	291 ± 10	0.50 ± 0.06
n	1.93 ± 0.13	1.57 ± 0.31	1.16 ± 0.04	1.00 ± 0.11	0.99 ± 0.03	1.13 ± 0.06
37°C						
K_i	14008 ± 5132	4440 ± 458	1709 ± 191	184 ± 22	347 ± 23	0.52 ± 0.08
n	2.06 ± 0.03	1.63 ± 0.25	1.21 ± 0.14	0.99 ± 0.06	1.00 ± 0.07	1.17 ± 0.11
ΔK_i	−11683	−2740	+741	+14	−210	−0.09
p	0.017	0.013	0.015	0.574	0.003	0.014

*
Standard control in all experiments and n = 21

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Table 2

Linear regression of the Van't Hoff relations and the estimation of $\Delta S°$ values [kJ/mol/K] and $\Delta H°$ values [kJ/mol]; T$\Delta S°$ [kJ/mol] and $\Delta G°$ values for T = 300 °K [kJ/mol]; data presented as (± SE) for fenoterol stereoisomers, (R)-isoproterenol and *rac*-propranolol

	(S,S')-fenoterol	(S,R')-fenoterol	(R,S')-fenoterol	(R,R')-fenoterol	(R)-isoproterenol	*Rac*-propranolol
R^2	0.926	0.921	0.858	0.204 [1]	0.844	0.919
Slope	4309 ± 703	2768 ± 469	−856 ± 201	---	2169 ± 537	542 ± 93
intercept	−2.52 ± 2.39	3.25 ± 1.59	16.03 ± 0.68	15.57 ± 0.1 [2]	7.84 ± 1.8	19.63 ± 0.32
$\Delta H°$	−35.8 ± 5.8	−23.0 ± 3.9	7.1 ± 1.7	0	−18.03 ± 4.5	−4.5 ± 0.78
$\Delta S°$	−0.021 ± 0.020	0.027 ± 0.013	0.133 ± 0.006	0.129 ± 0.001 [2]	0.065 ± 0.015	0.164 ± 0.002
$−T\Delta S°$	−6.3 ± 6.0	8.1 ± 4.0	40.0 ± 1.7	38.8 ± 0.2	19.5 ± 4.5	48.95 ± 0.79
$\Delta G°$	−29.5 ± 8.3	−31.1 ± 5.6	−32.9 ± 2.4	−38.8 ± 0.2	−37.6 ± 6.4	−53.5 ± 1.1

[1] – linear correlation not statistically valid so no slope reported (and assumed to be 0)

[2] – assuming no temperature dependence, the intercept value was calculated as the average of lnK_i values determined in different temperatures (± SD).