Biochemical Pharmacology 80 (2010) 1537–1545

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Towards a thermodynamic definition of efficacy in partial agonism: The thermodynamics of efficacy and ligand proton transfer in a G protein-coupled receptor of the rhodopsin class

Kenneth J. Broadley [a], Shane C. Sykes [a], Robin H. Davies [a,b,*]

[a] The Welsh School of Pharmacy, The University of Cardiff, PO Box 13, Cardiff CF10 3XF, Wales, UK
[b] Muscagen Limited, 10 North Road, Cardiff CF10 3DY, Wales, UK

ARTICLE INFO

Article history:
Received 28 June 2010
Accepted 21 July 2010

Keywords:
Thermodynamics
Efficacy
Agonist
Antagonist binding enthalpies
Ligand proton transfer
Rhodopsin receptors

ABSTRACT

The thermodynamic binding profiles of agonist and antagonist complexes of the 4-hydroxypropanolamine partial agonist, prenalterol, on the chronotropic adrenergic response in guinea-pig right atria were determined over a 15 °C temperature range. The tissue response was compared with data on the ethanolamine agonist, isoprenaline, given by binding studies in a number of rat tissues. Utilising the residue conservatism surrounding the known active conformers bound to either of two aspartate residues (α-helices II, III) in both receptors (β_1, β_2) and species (guinea-pig, rat and human), no significant deformation in the extended side chain could be found in prenalterol's agonist binding compared to isoprenaline. Antagonist binding gave a highly favourable entropy contribution at 30.0 °C of -4.7 ± 1.2 kcal/mol. The enthalpy change between bound agonist and antagonist complexes, a function of the efficacy alone, was -6.4 ± 1.1 kcal/mol, coincident with the calculated intrinsic preference of a primary/secondary amine–aspartate interaction for a neutral hydrogen-bonded form over its ion pair state, giving values of 6.3–6.6 kcal/mol with calculations of good quality, a figure expected to be close to that shown within a hydrophobic environment. Delivery of a proton to a conserved aspartate anion (α-helix II) becomes the critical determinant for agonist action with resultant proton transfer stabilisation dominating the enthalpy change. A proposed monocation-driven ligand proton pumping mechanism within the ternary complex is consistent with the data, delivery between two acid groups being created by the movement of the cation and the counter-movement of the ligand protonated amine moving from Asp 138 (α-helix III) to Asp 104 (α-helix II).

1. Introduction

An interpretation of partial agonism as the result of two bound complexes related to agonist and antagonist action existing within a G protein–receptor ternary complex was considered in paper I [1] and the requisite concentration–response theory for a compound obeying a hyperbolic relation derived using a simple adaptation of the Black and Leff operational model of agonism [2]. A tissue response, (the chronotropic in the guinea-pig right atrium) was selected based on data indicating its dominant control by the β_1-adrenergic receptor under normal conditions of signal amplification (refer also, paper I [1]). The advantage of such a description, transposing the observed parameters of binding and efficacy into two binding constants related to agonist and antagonist action, lies in temperature variation and the determination of the thermodynamic profiles of the respective agonist and antagonist complexes. Temperature studies [3–6] using isolated membranes for the binding of ligands to G protein-coupled receptors complexes utilising β-adrenergic receptor sub-types showed linear plots of unit slope over a 25 °C range of temperature offering the prospect of accurate enthalpic changes if sufficient precision were attained with a given receptor. While such data contain binding studies on more than one receptor sub-type, it was considered here, more illuminating to select a non-specific partial agonist structurally related to the natural hormone but to choose the tissue response dominated by a single receptor sub-type. Under normal signalling conditions, the chronotropic response in guinea-pig right atria was shown to be dominated by the β_1-adrenergic receptor with a less than 5% contribution from the β_2-receptor. Data 'in vitro' at 30.0 °C on the binding and efficacy of the phenoxypropanolamine partial agonist, prenalterol to this cardiac β_1-adrenergic receptor were given in the first paper [1]. The precision was shown to be sufficient for evaluation of the thermodynamics of the respective complexes over a 15 °C range of temperature [1].

* Corresponding author at: The Welsh School of Pharmacy, University of Cardiff, Redwood Building, King Edward VII Avenue, Cathays Park, Cardiff CF10 3DY, United Kingdom. Tel.: +44 0 2920 875830; fax: +44 0 2920 874149.
E-mail address: daviesrh@hotmail.com (R.H. Davies).

0006-2952/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2010.07.044

In this second paper, the binding and efficacy of prenalterol, are extended to 25.0 °C and 40.0 °C and the thermodynamic profiles of the agonist and antagonist complexes determined. The enthalpic difference between the agonist and antagonist complexes which is a function only of the efficacy, will be shown to have a simple physical interpretation consistent with a basic criterion for agonist action discussed below.

Considerable insight into structural features of ligands using close extra-thermodynamic comparison can be obtained by transforming data to a pure hydrocarbon phase reference using appropriate partitioning methods which can provide additional insight into the polar and distortional changes observable on receptor binding [7]. Using this reference, the thermodynamic profile of the agonist binding of prenalterol will be compared with published data for the non-specific 3,4-di-hydroxyphenethanolamine agonist, isoprenaline, determined from β-adrenergic receptors in a number of rat tissues. The β_1-receptor residues in guinea-pig, rat and human which surround the known bound ethanolamine conformer within 4.5 Å of its expected 'upper' (Asp138; α-helix III) and 'lower' (Asp 104; α-helix II) positions within the receptor are all conserved. Of the 19 residues in potential contact in the upper position and eighteen residues in the lower, only two residues in the upper position, Phe 362 (Leu-β_2) and Arg 208 (Asn-β_2) receptors distinguish β_1- from β_2-receptors. While these latter residues are not in immediate contact, their potential influence on signal amplification will be considered in Section 4.3. Selectivity in receptor binding is thus not expected with the ethanolamine derivative between rat, guinea-pig and human while consistency should also be observed in the binding of isoprenaline to β_1- and β_2-receptor derivatives in these three species. It will be later shown that the relevant agonist bound conformer of the phenoxypropanolamine derivative, prenalterol, surprisingly, shows no deformation in its binding to the β_1-adrenergic receptor but its thermodynamic profile is very closely related to the ethanolamine derivative. Finally, as reported earlier, [8] in comparison with data on membrane-binding studies, it is shown that the enthalpy associated with the Black and Leff amplifying parameter, τ, is negligible, indicative of a membrane diffusion process or ion channel opening.The publication of the first mammalian G protein-coupled receptor structure in 2000 [9] led to support for a proposed proton shuttle mechanism for the ligand action in an adapted G protein-coupled β_1-adrenergic receptor ternary complex with the identification of a series of potential proton relays in a GTP synthase mechanism of action [10]. More recently, the adapted receptor structure has been confirmed by the presence of very similar α-helical packing in a modified β_2-adrenergic receptor structure [11]. We present a simple physical interpretation of the enthalpic changes observed between agonist and antagonist complexes supporting the view that minimal agonist response is dependent on ligand protonation of the conserved aspartate residue at position 104 on α-helix II. This is consistent with the wider proposed mechanism of the operation of the ligand as a proton shuttle operating on delivering proton transfer through two proton relays under the driving force of a monocation.

2. Materials and methods

Experimental protocols for determining binding constants and efficacies of the partial agonist, prenalterol [3-(4-hydroxyphenoxy)-1-isopropylamino propan-2-ol, refer Fig. 2], based on the β_1-adrenoceptor dominating the chronotropic response in guinea-pig right atria were given in Paper I [1]. The simple conversion to agonist and antagonist binding complexes was exemplified with data at 30.0 °C.

3. Results

The conformer binding constants are closely related to the Black and Leff representation and are summarised in (1). The apparent binding constant K'_B of the partial agonist B is the sum of the conformer binding constants but where the agonist component incorporates an amplification factor τ from the signal enhancement of the response. The agonist component may thus be defined by $K^{ijx}(\tau+1)$ where the agonist conformer i interacts with its receptor counterpart j^x but there is an additional term $K^{ijx}\,\tau$ from the signal amplification. The antagonist component is defined by the ligand and receptor conformers r and s^x respectively or some appropriate sum. The observed conformer binding constants are very simply related to the apparent binding constant K'_B by the relations

$$\begin{aligned}(1-e_B)K' &= K^{rsx}\\ e_B K' &= K^{ijx}(\tau+1)\end{aligned} \qquad (1)$$

and the appropriate free energy relationships may be defined. As commented earlier, the fact that such bound drug–receptor conformers may have no separate unbound existence does not invalidate their pharmacological definition and thermodynamic functions can obviously be associated with such forms. The theoretical unbound equivalent forms, however, represent the ideal conformation for maximum interaction with the receptor in the given binding mode. In these simple functions, it is seen in (1) that the ratio of the two conformer constants is independent of the apparent binding and is a function of the efficacy alone. Thus on the log scale, differences in these conformer constants plotted as a reciprocal function of the absolute temperature, or more simply changes in the efficacy function alone yield the required enthalpy changes in going from the bound antagonist to the agonist form directly. This is a remarkably useful result in elucidating the contributions to the composite energy requirements for receptor activation.

Table 1
Apparent binding constants (K'_B), efficacies (e_B/e_A), antagonist (K^{rsx}) and agonist ($K^{ijx}(\tau+1)$) binding components of prenalterol at 40.0 °C on the guinea-pig cardiac β_1-adrenoceptor.

Efficacy e_B/e_A	EC_{50} measurements				Ferguson–Robertson				Waud $\log_{10}K^{rsx}$
	$\log_{10}K'_B$	$\log_{10}K^{rsx}$	$\log_{10}K^{ijx}(\tau+1)$	Langmuir slope	$\log_{10}K'_B$	$\log_{10}K^{rsx}$	$\log_{10}K^{ijx}(\tau+1)$	Langmuir slope[a]	
0.37	7.18	6.98	6.75	0.69 ± 0.02(4)	7.29	7.09	6.86	1.20 ± 0.04(3)	6.65 ± 0.05
0.40	7.02	6.80	6.62	1.06 ± 0.08(4)	7.40	7.18	7.00	0.89 ± 0.03(3)	6.67 ± 0.08
0.42	7.09	6.85	6.71	0.71 ± 0.21(3)	7.31	7.07	6.93	1.10 ± 0.03(3)	–
0.44	7.31	7.06	6.95	0.90 ± 0.02(3)	7.40	7.15	7.04	0.89 ± 0.04(4)	6.99 ± 0.03
0.50	7.09	6.80	6.78	0.90 ± 0.03(3)	7.05	6.75	6.75	0.98 ± 0.04(4)	6.72 ± 0.09
0.53	7.27	6.94	6.99	0.78 ± 0.02(3)	7.11	6.78	6.81	1.04 ± 0.08(4)	6.80 ± 0.09
Mean ± SEM									
$0.44_2 \pm 0.02_4$	$7.16_0 \pm 0.04_6$	$6.90_5 \pm 0.04_3$	$6.80_0 \pm 0.05_8$	0.84 ± 0.06(6)	$7.26_0 \pm 0.06_0$	$7.00_3 \pm 0.07_7$	$6.90_0 \pm 0.04_5$	1.02 ± 0.05(6)	$6.76_6 \pm 0.06_1$(5)

[a] Slope of Langmuir relation for $R(A/B)$.

Table 2
Apparent binding constants (K'_B), efficacies (e_B/e_A), antagonist (K^{rsx}) and agonist ($K^{ijx}(\tau+1)$) binding components of prenalterol at 25.0 °C on the guinea-pig cardiac β_1-adrenoceptor.

Efficacy e_B/e_A	EC_{50} measurements				Ferguson–Robertson				Waud $\log_{10} K^{rsx}$
	$\log_{10} K'_B$	$\log_{10} K^{rsx}$	$\log_{10} K^{ijx}(\tau+1)$	Langmuir slope	$\log_{10} K'_B$	$\log_{10} K^{rsx}$	$\log_{10} K^{ijx}(\tau+1)$	Langmuir slope[a]	
0.41	7.45	7.22	7.06	$0.87 \pm 0.10(4)$	7.55	7.32	7.16	$0.86 \pm 0.04(6)$	6.98 ± 0.12
0.45	7.46	7.20	7.11	$0.92 \pm 0.20(3)$	7.46	7.20	7.11	$1.16 \pm 0.09(5)$	7.00 ± 0.10
0.51	7.46	7.15	7.17	$1.04 \pm 0.10(3)$	7.58	7.27	7.29	$1.05 \pm 0.07(5)$	7.13 ± 0.06
0.52	7.35	7.03	7.07	$1.06 \pm 0.05(4)$	7.52	7.20	7.24	$0.94 \pm 0.07(3)$	6.96 ± 0.12
0.60	7.44	7.04	7.22	$1.10 \pm 0.06(3)$	7.35	6.95	7.13	$0.99 \pm 0.07(5)$	6.62 ± 0.11
0.64_5	7.63	7.18	7.44	$0.84 \pm 0.06(3)$	7.40	6.95	7.21	$1.10 \pm 0.03(4)$	6.78 ± 0.11
0.78	7.39	6.73	7.28	$0.92 \pm 0.09(4)$	7.62	6.96	7.51	$1.36 \pm 0.18(4)$	–
Mean ± SEM									
$0.55_6 \pm 0.04_4$	$7.45_4 \pm 0.03_3$	$7.07_9 \pm 0.06_5$	$7.19_3 \pm 0.05_1$	$0.96 \pm 0.04(7)$	$7.49_7 \pm 0.03_7$	$7.12_1 \pm 0.05_2$	$7.23_6 \pm 0.05_2$	$1.07 \pm 0.06(7)$	$6.91_2 \pm 0.07_4(6)$

[a] Slope of Langmuir relation for $R(A/B)$.

The respective binding constants at 40.0 °C and 25.0 °C are given in Tables 1 and 2. Over the narrow 15 °C temperature range, the Langmuir behaviour is observed, the slopes of the dose–response relations for both the agonist and antagonist responses of prenalterol having slope relations not significantly different from unity. This is also shown for the data at 30.0 °C in the first paper [1]. For individual experiments at 40.0 °C and 25.0 °C, there is a slight, but not significant tendency for experiments with lower efficacy to have apparent binding constants (K'_B) slightly greater when determined by antagonist binding than by agonist binding.

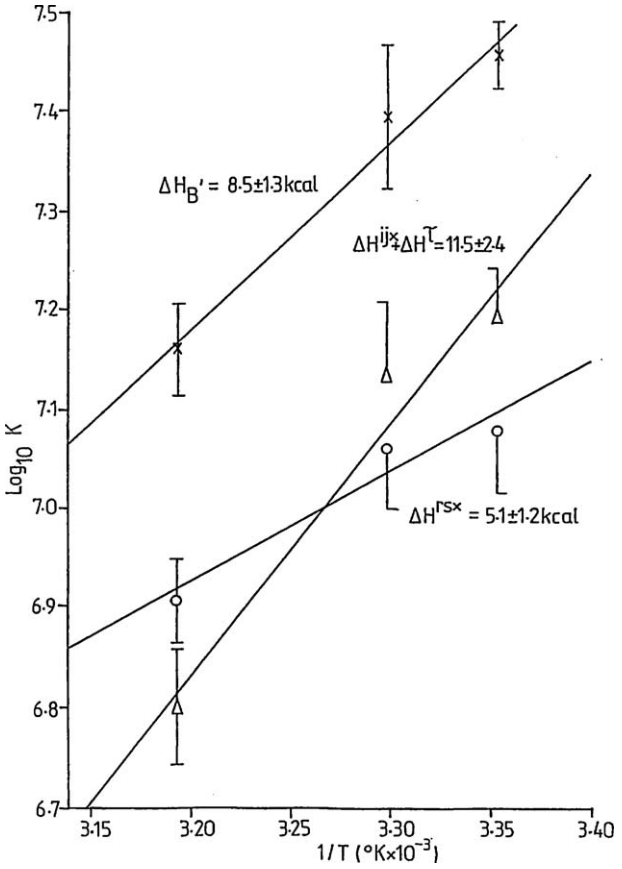

Fig. 1. Enthalpies of binding of agonist and antagonist components of the binding of prenalterol on the guinea-pig cardiac β_1-adrenergic receptor. Plot of agonist and antagonist conformer binding constants on the $\log_{10}$ scale plotted against $1/T$ where T is the absolute temperature. The binding constants shown here are determined from the agonist response (EC_{50} values). The enthalpy of the overall binding constant without separation into components (8.5 ± 1.3 kcal/mol) is also shown. The enthalpic difference between the bound agonist and antagonist complexes fitted as a function of the efficacy, e alone gave a value of 6.4 ± 1.1 kcal/mol.

The observed binding constants whether determined from the agonist or antagonist response of the partial agonist show no significant differences over the three temperatures (40.0 °C 7.16 ± 0.05 (6), (EC_{50}); 7.26 ± 0.06 (6), (F–R): 25.0 °C 7.45 ± 0.03 (7) (EC_{50}); 7.50 ± 0.04 (7) (F–R)) indicating, as expected, a competitive form of signal amplification of the response as exemplified in the first paper. Accuracies in binding and efficacy on the $\log_{10}$ scale are required to within $\pm 0.03_5$ $\log_{10}$ units for determining the enthalpy of binding between agonist and antagonist conformers to within 1.0–1.5 kcal/mol over the 15 °C temperature range and are of this order. Plotting of the EC_{50} binding data against the reciprocal of the absolute temperature with the associated enthalpies of interaction are shown in Fig. 1. The data have very slightly tighter error bars compared with the data reported in our 1991 paper [8] but are, in effect, identical to the reported values (±0.1 kcal/mol). The earlier thermodynamic enthalpic functions are reproduced in Table 3. The binding constants determined from the blocking action of the partial agonist have tighter error bars but the differences between the conformer binding enthalpies are, unsurprisingly, the same 6.6 ± 1.6–2.7 kcal/mol. Direct fitting of $e/(1-e)$ against $1/T$ on the log scale gives an enthalpy of activation of 6.2 ± 1.1 kcal/mol [8]. We take a compromise figure of 6.4 kcal/mol for this activation of agonist action for prenalterol and compare existing data on the thermodynamics of binding of ethanolamine and phenoxypropanolamine agonists and antagonists on the β_1-adrenergic receptor.

4. Discussion

4.1. Extra-thermodynamic comparison of related derivatives of prenalterol and the use of a reference hydrocarbon phase model

There is a wealth of evidence to suggest that the best insight into comparing binding of ligands within membrane-bound proteins is to employ a mobile reference hydrocarbon phase model. At the membrane level, the relation between observed binding and that predicted by a partitioning effect into a hydrocarbon phase is one of exact unit slope where no change in intrinsic binding takes place [12]. The question arises as to how far extra-thermodynamic changes can be usefully interpreted by such a model when comparing closely related species and their

Table 3
Comparison of enthalpic changes associated with agonist and antagonist conformer binding of the 4-hydroxyphenoxypropanolamine partial agonist, prenalterol, to the guinea-pig cardiac β_1-adrenergic receptor. Determinations utilising agonist (EC_{50} values) and antagonist (Ferguson–Robertson) responses from 40.0 °C to 25.0 °C [8].

	EC_{50} (kcal/mol)	Ferguson–Robertson (kcal/mol)
ΔH observed	-8.6 ± 1.9	-8.0 ± 0.2
ΔH^{rsx}	-5.0 ± 0.9	-4.2 ± 0.6
ΔH^{ijx}	-11.6 ± 2.7	-10.8 ± 1.5

Table 4
Incremental thermodynamics of partitioning for the $-CH_2-$ group [24].

Partitioning phases	310 K	kcal/mol	
	$\delta\Delta G$	$\delta\Delta H$	$-T\delta\Delta S$
1. Cyclohexane/gas	−0.76	−1.12	+0.36
2. H_2O/gas	+0.18	−0.67	+0.85
Cyclohexane/H_2O	−0.94	−0.45	−0.49

thermodynamic changes bearing in mind the overall accuracy required in observing thermodynamic changes. An early exploration showed that such incremental enthalpic and entropic changes could be followed for simple alkyl substitution on the ligand where the free energy changes indicated that there was flexible freedom for localised groups even when adjacent to expected polar ligand-receptor interactions in mammalian β-adrenergic receptors. The data were exemplified by predicting the thermodynamic binding changes of adrenaline from isoprenaline based on simple alkyl differences observed in turkey erythrocyte β-adrenergic receptor data [7]. The underlying reason for predicting these changes is based on the incremental thermodynamics of partitioning of the $-CH_2-$ group where the local interactions in hydrocarbon and water are energy and entropically driven respectively and the data are reproduced in Table 4 [13]. The overall partitioning change from water to hydrocarbon becomes one of apparent simplicity, the overall effect being one of almost equal favourable enthalpic and entropic ($-T\Delta S$) contributions of −0.45 kcal/mol and −0.49 kcal/mol respectively at blood temperature, with an overall group free energy change of −0.94 kcal/mol. The effective overall group partitioning assumption on the basis of simple additivity appears accurate to within 5% in accordance with conclusions attributable to three body effects in solution in simple non-polar inert gas liquid mixtures [14]. A group additivity assumption directly applied to the potency of halogenated hydrocarbons as volatile anaesthetics perturbing an ion channel was accurate to 5–10% [15,16]. Thus, if there is regional tolerance in the binding of the alkylamino groups in isoprenaline and adrenaline we should expect a free energy increment at 30.0 °C of −1.84 kcal/mol with appropriate local enthalpic and entropic contributions.

We use this simple group assumption to check the consistency of earlier reported thermodynamic binding data on isoprenaline and adrenaline on rat β-adrenergic receptors within the cerebral cortex, cerebellum, heart and lung, where the cortex and heart are dominated by β_1-adrenergic receptors and the cerebellum and cortex predominantly by the corresponding β_2-receptors [2]. The data are confined to those where 300 μM concentrations of GTP were added. As commented in Section 1, early structural models of the β_1- and β_2-adrenergic receptors based on bovine rhodopsin (refer Section 6) show that the immediate residues surrounding these two ligands, isoprenaline and prenalterol bound to the two conserved aspartate residues on α-helices III and II in the respective binding sites are identical both for rat, guinea-pig and human. Table 5 shows the accurate consistency of these data at the membrane binding level when comparing isoprenaline (observed) with isoprenaline (predicted) from adrenaline. The adrenaline lung datum was omitted on account of its large mean error in the binding, being an order of magnitude greater than the average. The mean enthalpic change on binding over the four

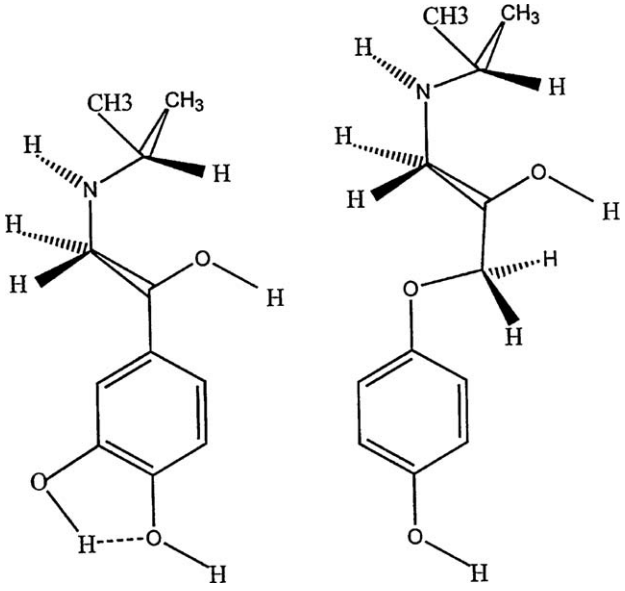

Fig. 2. Comparison of the known agonist side-chain conformer of isoprenaline (a) bound to the cardiac β_1-adrenoceptor [17] compared with the expected bound conformation of the prenalterol (b) based on the almost identical enthalpies of binding. Less relevant hydrogen atoms are only indicated directionally.

Table 5
Comparison of equilibrium thermodynamic parameters of the agonist component of prenalterol on rat β-adrenergic receptors at 30.0 °C with experimental and predicted values for isoproterenol (isoprenaline) (predictions based on the close analogue L-epinephrine (adrenaline) see text and [7]). Determinations and predictions for isoproterenol made with +300 μM GTP present.

	ΔG^0 (kcal/mol)	ΔH^0 (kcal/mol)	$T\Delta S^0$ (kcal/mol)
Cerebral cortex			
l-Isoproterenol (exptl)	−9.4 ± 0.06	−12.5 ± 0.48	+3.1 ± 0.54
l-Isoproterenol (predicted)	−9.4 ± 0.06	−12.6 ± 0.48	+3.2 ± 0.54
Cerebellum			
l-Isoproterenol (exptl)	−9.5 ± 0.05	−11.3 ± 1.30	+1.8 ± 1.35
l-Isoproterenol (predicted)	−9.8 ± 0.04	−11.9 ± 0.83	+2.1 ± 0.87
Heart			
l-Isoproterenol (exptl)			
l-Isoproterenol (predicted)	−9.4 ± 0.05	−11.7 ± 0.41	+2.3 ± 0.46
Lung			
l-Isoproterenol (exptl)	−10.7 ± 0.03	−10.2 ± 0.55	−0.05 ± 0.548
l-Isoproterenol (predicted)	−9.1 ± 0.3[a]	−7.8 ± 0.98	−1.3 ± 1.02
Mean (6)	$-9.7 \pm 0.2_1$	$-11.7 \pm 0.3_6$	$+2.0 \pm 0.5_4$
l-isoproterenol (tissue average)			
Prenalterol agonist component	−9.9 ± 0.10	−11.5 ± 2.4 $\Delta H^{ijx} + \Delta H^{\tau}$	+1.6 ± 2.4
Antagonist component	−9.8 ± 0.10	−5.1 ± 1.2 ΔH^{rsx}	−4.7 ± 1.2

[a] Rejected on SEM.

tissues gives $-11.7 \pm 0.3_6$ kcal/mol over six of the experimental and predicted data points. These experimental and predicted estimates provide a convenient test of potential errors as we have omitted the direct experimental evidence for isoprenaline on the dominant cardiac β_1-adrenergic receptor where the enthalpy change is reported to be -17.2 ± 1.03 kcal/mol. We have no explanation for why this result for the cardiac receptor should show such a significant difference from the predicted value based on adrenaline in these otherwise excellently consistent data based on binding alone, particularly when simple correlations at the free energy level 'in vitro' on the rat and higher species indicate tolerance of the methyl and isopropyl non-polar groups within the β_1-receptor site [5]. Structurally and experimentally, the ligands, themselves are not expected to show tissue selectivity between β_1- and β_2-receptor binding nor to exhibit binding differences in common species. The data require further validation but we take the hypothesis that the most consistent values for the thermodynamics of binding of isoprenaline to the cardiac β_1-receptor are likely to be those given by the mean values in Table 5. This assumption must remain a working hypothesis.

4.2. Binding thermodynamics of the agonist conformer of prenalterol

Thermodynamic data on the agonist binding of the phenoxypropanolamine derivative, prenalterol (refer also Table 5), determined from chronotropic reponses are in very close agreement with those predicted from the mean values of the ethanolamine analogue, isoprenaline, using radioligand binding studies. This is an unexpected result and we first review any concentration difference expected from extra-thermodynamic comparison using a hydrocarbon reference phase. The two compounds are shown in Fig. 2. In the agonist conformer [17], the 3-hydroxy group of isoprenaline is expected to have an intramolecular hydrogen bond to the 4-oxygen atom while both compounds have two delocalised aromatic oxygen atoms. For any concentration difference in a hydrocarbon reference phase, this difference on the free energy scale is expected, at most, to be of the order of 0.5–0.9 kcal/mol. We take this value to be not significant for comparison purposes. Our first conclusion is that, since the rat thermodynamic data were determined from radioligand membrane binding studies, there is clear evidence in prenalterol activation that the enthalpy change associated with the signalling amplification factor, τ, is very small. This is indicative, as previously commented, of a diffusion process from ion channel opening rather than a specific activation energy.

The current background to the known binding conformations of phenoxypropanolamine and phethanolamine ligand side chains is reviewed. Much of the development of the understanding of the behaviour of ligands in the β_1-adrenergic receptor site has come from close molecular comparison and synthetic effort. The agonist conformation of the adrenaline side chain was determined by appropriate cyclisation of the ethanolamine side chain and the resultant identification of the agonist conformer [17]. Cyclisation of the side chain of the appropriate phenoxypropanolamine derivative to fold the amino group back to an ethanolamine position proved inactive in both agonist and antagonist action (Clarkson R, Dowell R, unpublished data). These data suggested that a possible contraction of the phenoxypropanolamine side chain closer to the given ethanolamine agonist conformation might be the primary cause of agonist action. Some support for this came from a series of 2-substituted phenoxypropanolamine analogues of prenalterol lacking the p-hydroxyl substituent which showed a constant observed binding constant ($K'_B \pm 0.15$ kcal/mol) when referenced to the hydrophobic environment but the efficacy varied between 0.13 and 0.51 [18]. The energetics of an in-plane distortion around the 2-substituted phenoxymethylene moiety

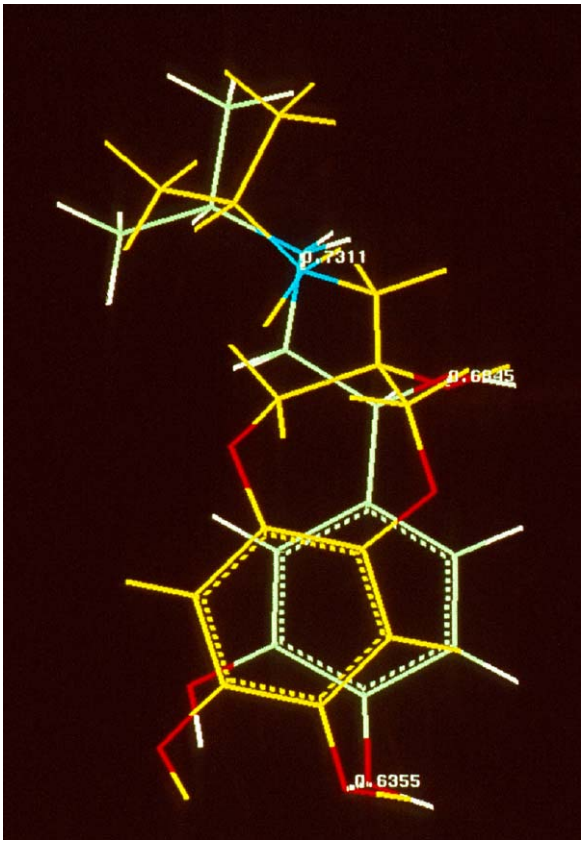

Fig. 3. Comparison of intermolecular distances in molecular overlap between the predicted agonist conformers of the benzdioxepine analogue and isoprenaline [18].

consistent with a small contraction of little more than 0.5 Å gave a correlation of unit slope when plotted against the hyperbolic efficacy relation on the thermodynamic scale over a predicted 1.2 kcal/mol range. This was consistent with the highest observed agonist activity of a phenoxypropanolamine derivative in the form of a benzdioxepine substituent with the appropriate side chain (see Fig. 3). On the free energy scale, the movement from antagonist to almost full agonist over a 5–95% efficacy range should be complete over a 2.0 kcal/mol free energy range and these energetics for close comparison were of the right order. Yet we have a large enthalpic change in going from an antagonist to agonist complex within the phenoxypropanolamine agonist complex which is, as yet, unaccountable.

The almost exact correspondence of the binding thermodynamics of the extended agonist conformer of the phenoxypropanolamine partial agonist, prenalterol, with those for the phenethanolamine full agonist, isoprenaline, together with the similar reference concentrations anticipated within the mobile reference hydrocarbon phase of the protein, indicate that we cannot argue about further distortion within the bound phenoxypropanolamine agonist conformer. The ligand–receptor interactions from the para-hydroxyl group and from the basic side chain of the ligand in agonist action appear very closely similar to those of the ethanolamine derivative and must be very close to optimal with an extended phenoxypropanolamine side chain bound conformer. Any conformational change must, therefore, come from a facile movement of a receptor residue. The obvious movement in the context of agonist action which is later discussed, is that of a dihedral rotation of the conserved aspartate residue (α-helix II) from its expected ethanolamine interaction of -60.0°, to an extended 180.0° giving a minimal increased separation between relevant oxygen atoms of 2.6 Å sited along a similar vertical axis

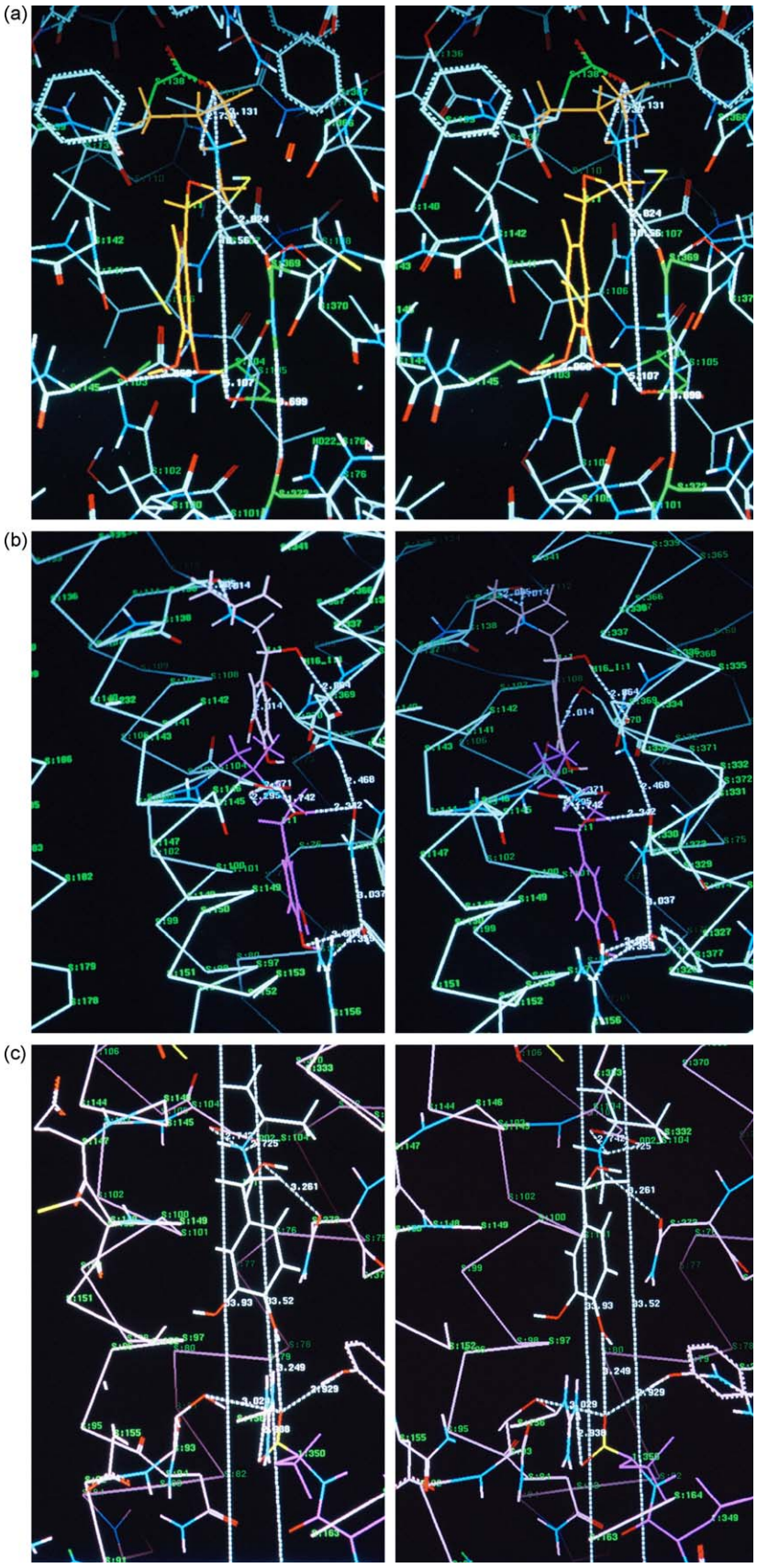

Fig. 4. (a) CHARMm minimised energy structures [36] (using a hydrophobic dielectric of 4.0 and hydrogen bond constraints with the protein backbone fixed) for the agonist conformer of the ligand, isoprenaline (1-R [3,4-di-hydroxyphenyl] 2-isopropylamino ethan-1-ol), binding in two possible positions to the aspartate residues on α-helices III and II respectively [10]. The G protein and cytoplasm lie to the base of the figures. (a) In the upper position, the basic ligand (brown) binds to Asp 138 (III). The agonist conformer is dictated by the β-hydroxyl group's interaction with Asn 369 (VII) and is closely maintained on minimisation. This residue, together with Asn 373 moves to a more vertical orientation relative to the membrane. The potential ligand-binding residues are shown in green. (b) Comparison of the CHARMm energy-minimised agonist

across the membrane-bound protein. Here the extended conformer of the phenoxypropanolamine compound can interact through its basic amino group with the aspartate residue but without significant energy loss in the form of deformational energy along the molecular side chain.

An early examination of a contraction in the phenoxypropanolamine side chain of a β_1-adrenergic receptor partial agonist attempted to see whether inhibition of vibrational contributions in the side chain could contribute significantly to the energetics observed for contractions involving 0.2 Å, 0.5 Å and 1.0 Å respectively in an approximate 1-dimensional contraction of the bound molecule retaining the expected agonist conformer [19]. With a limited basis set, the scale of these unfavourable deformation energies gave 0.4 kcal/mol, 1.5 kcal/mol and 4.6 kcal/mol respectively. The vibrational energy differences on contraction were, however, negligible. In assessing the potential contributions, we utilised the observed enthalpy of binding for isoprenaline on the cardiac β_1-adrenergic receptor taking the previously discussed value of 17.2 kcal/mol and attempted to allocate a compensatory contraction as a component of the value. This hypothesis is now rejected and we retain the mean tissue enthalpic change of $-11.7 \pm 0.3_6$ kcal/mol for the binding of isoprenaline to the rat cardiac β_1-adrenergic receptor as discussed above.

The very significant enthalpic difference of -6.4 ± 1.1 kcal/mol observed between agonist and antagonist complexes may now be considered. One might, in general, have expected quite close enthalpic differences between the two complexes for a 50% partial agonist reaching a critical activation energy for achieving a response or just failing to do so unless some critical stabilisation energy was invoked before activation of the receptor became possible. A critical feature of agonist action in the proton pumping mechanism is the delivery of a proton from the basic protonated amino side chain of the ligand to the receptor's aspartate 104 anion to form the more stable neutral hydrogen bonded acid–base with the resultant delivery of the proton to the receptor. The criterion for stimulant action at its most basic level would be, therefore, the delivery of a proton to this residue in the receptor where an automatic stabilisation to the neutral hydrogen bonded acid–base form would occur. This is strongly supported by the intrinsic stabilisation energy between the neutral and ion pair forms of the acid–base interaction [10]. In Table 1 of this reference, three extensive calculations including higher level electron and exchange correlation contributions and a density functional comparison (HF 6-31 + +G** 6.6; +MP2 6.2; B3-LYP 6.3 kcal/mol) showed strong support for this stabilisation energy which in a hydrophobic environment is anticipated to be close to this value. Thus arrival of the protonated amine in a correct orientation for proton transfer to this aspartate 104 anion site automatically creates this additional stabilisation energy on transfer of the proton and would provide the most basic criterion for agonist action, namely that a proton must be resident at Asp 104 for agonist action (with subsequent delivery to the Gα-protein under the influence of a monocation).

4.3. The ligand–receptor–G protein ternary complex and signal amplification

The development of a monocation-driven proton pump within the ligand–receptor–G protein-ternary complex where the ligand acts as a proton shuttle has developed quite simply from these thermodynamic considerations on the binding of the partial agonist, prenalterol and its companion ethanolamine derivative, isoprenaline, taken in conjunction with the adapted β_1-adrenergic receptor structure and its proposed ternary complex, based on mammalian bovine rhodopsin [10]. Prior to the publication of the bovine rhodopsin structure, a steady state model of the ligand–receptor–G protein complex functioning as a GTP synthase in the β_1-adrenergic receptor complex was explored [20]. While the proposed acid–base–acid shuttle was not correct involving supporting tyrosine residues rather than more readily ionised acid groups, the exploration of the steady state equations using the ternary complex format [21,22] had widespread generality. The model could not distinguish between a GTP synthase or a GDP/GTP exchange model on receptor stimulation. The steady state conditions led to a number of potential solutions with forty potential terms in the denominator, but two dominant features leading to a hyperbolic form of the dose–response were cited. The steady state controlling rate was (1) rate limiting due to activation of proton signalling through an initiating acid–base–acid triad or (2) rate controlled by G protein release but there was, also, an activation barrier to proton signalling though the initial residue triad. The terminology is slightly different to that employed here but is closely correspondent. The approximate steady state solutions for (1) and (2) are very similar but there is an additional multiplicative rate constant, k_{31}, in the latter, being the effective rate of G protein release. The absence of an enthalpic energy associated with the signal amplification factor, τ indicates (1) that there is a likely diffusion process activating the signal mechanism and possibly, also, for G protein release or (2) the release of the G protein is not rate limiting. But do we expect to see signal amplification in a steady state solution for proton pumping where there is a 1:1 synthesis of GTP from GDP due to proton-activated phosphate release from a glutamate residue at the base of the G_α α2-helix (at Glu 203) with transfer to form GTP followed by a 1:1 formation of cAMP by activation of the adenylyl cyclase by the energised $G_\alpha s{\cdot}GTP$ protein? An early study showed an absence of signal amplification in the production of cAMP from isoprenaline [23]. This conclusion should be valid for any common process of signal amplification involving the $G_\alpha s$ protein. Equilibrium binding constants for isoprenaline based on cAMP accumulation gave mean values -7.13 ± 0.09 on the $\log_{10}$ scale from 2 laboratories [24–26]. Seven binding studies using guinea-pig and cat with both atria and ventricle and rat and dog with ventricle alone, gave an average binding constant on this scale of $-6.8_3 \pm 0.1_3$ (omitting two large outliers) [27]. Table 2 radioligand binding studies for the three experimental values of isoprenaline and three predicted results (based on l-adrenaline) produced an average value of -7.0 ± 0.15. Binding constants observed from chronotropic responses, on the other hand, show observed values of $-8.9_0 \pm 0.1_1$ [28,29], suggesting an amplification factor of 50–100 and a similar result exists for the inotropic response $-8.8_7 \pm 0.1_8$ on the $\log_{10}$ scale [30]. However, in Table 2, the thermodynamic parameters for prenalterol were determined from the chronotropic response but compared with the values for isoprenaline determined from radioligand binding studies.

The key to this apparent anomaly lies simply in the effective value of τ for a partial agonist. It may be seen from Eq. (1) that by the definition of the partial agonist, the net product

conformer when bound at Asp 138 (pink) and, in its lower position, when interacting with Asp 104 (purple) on helix II. The agonist conformer in the latter position is again closely maintained on energy minimisation with the β-hydroxyl group now interacting with Asn 373. The dihedral controlling the lower aspartate 104 interaction is close to −60.0°. (c) CHARMm energy-minimised interaction of the bound agonist conformer with the amine moiety bound to Asp 104 (II – rear) with the p-hydroxyl moiety interacting with the proposed Gsα 350 terminal acid group now held by the receptor's Arg 156 (III – foreground) and Tyr 377 (VII). The 33.7 Å distance to the rear of the figure represents the interatomic distances between oxygen atoms of Asp 138 (III) and Gsα Asp 213 at the top of the Gsα α2-helix. On interaction of the ligand p-hydroxyl moiety it is proposed that the Arg 156 residue is released, moving to the intramolecular Asp 155. The proposed shuttle mechanism of the ligand under the influence of a monocation is outlined in [10].

$K^{ijx}(\tau+1) \sim K^{rsx}$ when the efficacy is in the region of 0.5 and the overall error in the true binding constant is, at most, within a factor of 2 of its accurate value. Similarly, this effective value of τ should be defined by the explicit steady state equations for proton pumping [20]. In conditions (12) and (13) of the paper (condition A1) where there is an activation barrier to proton signalling through the initiating residue triad, there is an explicit activation rate, k_{act1} following the binding of the agonist conformer K^{ijx} but the terms are, otherwise, closely correspondent. The dominant terms for the linear response may be written

$$\frac{[X^*]}{[R_{TOT}]} = \frac{k_{act1}}{k_e}\frac{K^{ijx}[A]}{1+K_A[A]} = \tau_A\frac{K^{ijx}}{K_A}\frac{K_A[A]}{1+K_A[A]} \quad (2)$$

where k_e is the effective rate of conversion of the activated adenylyl cyclase or proportional to it.

For the hyperbolic response

$$\frac{[YX^*]}{[R_{TOT}]} = \tau_A\frac{K^{ijx}[A]}{1+\tau_A K^{ijx}[A]+K_A[A]} = \tau_A\frac{K^{ijx}}{K'_A}\frac{K'_A[A]}{1+K'_A[A]} \quad (3)$$

where $K'_A = K_A + \tau K^{ijx} = K^{rsx} + K^{ijx}(\tau+1)$, the apparent binding constant containing the amplification term τK^{ijx}. If τ_A is scaled by $\tau_A + 1$ for the hyperbolic response, the efficacy is as before.

The absence of an enthalpic change associated with the factor τ led to a search for potential data on related ion channels. Evidence that the local opening of a monocation channel on a rabbit atrial cell on stimulation by acetylcholine has been known for the last twenty-five years [31,32]. The current was shown by patch pipette methods where the pipette, containing isotonic potassium chloride, holds the rabbit atrial cell in a surrounding bath. When the acetylcholine is in the bath no increase in current is seen (where release of a G protein might migrate to the region of the patch). The current was thus shown to be G protein dependent but no second messenger was involved. It was proposed that a directmodulation of a potassium (Ach) channel was involved in this membrane-delimited signalling, the activated G protein interacting directly with the channel. The presence of sodium ion has, also, been shown essential at Asp 104 on α-helix II for agonist action [33–35]. The obvious hypothesis is that the incipient monocation channel associated with receptors of the rhodopsin class could reside within the receptor-G protein complex and be based on acid groups on α-helices I, II, III and VII. An attractive feature of the developed ternary complex model was that three acidic residues Asp 212, Cys 209 and Glu 203 of the G_αs α2-helix could be aligned with conserved acidic residues of the receptor and at similar inter-residue distances in the overall pathway to the cytoplasm's Glu 203, a pathway showing a verticality to the expected membrane surface and closely parallel to the orientation of α-helix IV. The precise mechanism based on neutral acid–base interactions and their conversion to ion-pair forms under the influence of the monocation and resultant movement of the protonated base, has already been cited. Fig. 4 reproduces the known agonist conformer of isoprenaline binding in its upper and lower positions to the two aspartate groups on α-helices III and II at Asp 138 and 104 respectively.

A final word on the Black and Leff amplification factor, τ, is appropriate. It may be seen that, at the receptor level two methods of exploitation of compound selectivity of action are achievable. Firstly a direct exploitation of residue binding differences but secondly small perturbations to the proton transfer pathway may also create selective action by perturbing the efficiency of the proton transfer. Both mechanisms imply some variation in residue or selective binding but the latter action may prove relatively indirect.

The findings of this thermodynamic study on partial agonism may be summarised.

1. An adaptation of the Black and Leff operational model of agonist action in receptor theory where a single parameter, τ, is sufficient to define an amplifying contribution to any dose–response relationship exhibiting a hyperbolic form has been found useful in a treatment of partial agonism. By transposing efficacy and gross binding into two effective conformer binding components related to agonist and antagonist action, the binding thermodynamics of the two components of the partial agonist, prenalterol on the β_1-adrenergic receptor were determined.
2. The thermodynamic binding profile for the agonist component of the binding of the 4-hydroxyphenoxy-propanolamine, prenalterol, to the dominant cardiac β_1-adrenergic receptor determined from chronotropic responses in guinea-pig right atria, was almost identical to that shown by the 3,4-dihydroxyphenethanolamine derivative, isoprenaline binding but based on radioligand binding studies in a number of tissue receptors in the rat. Since the amino acids in guinea-pig, rat and human in the immediate proposed binding regions of the two bound agonist ligands are all conserved in both β_1- and β_2-receptors, there is clear evidence that no deformational differences in binding can be present within the agonist bound conformers of the two compounds. The ligand–receptor interactions anticipated for the p-hydroxyl and side chain amino groups are concluded to be effectively identical. This is only achievable in the expected agonist receptor site by facile movement of a receptor residue, the candidate being the conserved Asp 104 on α-helix II, the dihedral rotation from -60.0° to 180.0° giving a minimal increased separation between relevant oxygen atoms of 2.6 Å sited along a similar axis across the membrane-bound protein.
3. The enthalpic component of the amplifying factor, τ, was concluded to be negligible, the amplification being suggestive of a diffusion process or ion channel opening.
4. The temperature dependence of the efficacy ratio $e/(1-e)$ on the thermodynamic scale yielding the enthalpic difference between the agonist and antagonist complexes directly, gave a value of -6.4 ± 1.1 kcal/mol, a value highly consistent with the intrinsic stabilisation energy of a neutral hydrogen bonded acid–base over its ion pair form. This calculated value of 6.2–6.6 kcal/mol for the equivalent of a basic primary alkylamino-aspartate interaction, is expected to be close to that in a hydrophobic environment. The stabilisation energy between agonist and antagonist complexes can, therefore, be consistent, with the delivery of a ligand proton to the aspartate ion at residue 104 defining the minimal critical determinant of agonist action.
5. Signal amplification expected from the chronotropic response associated with the cardiac receptor used for determining the binding constant 'in vitro' was not found. The apparent absence of amplification when compared with data on radioligand binding studies arises from the nature of partial agonism, there being, in the component conformer representation, equal contributions of the antagonist and amplified agonist contribution when the efficacy is 0.5. Thus any error in the estimate of total binding arising from agonist signal amplification can, at most, be a factor of two only.
6. The high favourable entropy of binding of the antagonist conformer with a $-T\Delta S$ contribution of some -4.7 ± 1.2 kcal/mol at blood temperature is consistent with data on other phenoxypropanolamine antagonists on the β_1-adrenergic receptor.

Acknowledgements

The authors would like thank Dr. J. D. Fitzgerald for his support and scientific encouragement and acknowledge the support given to the work by ICI under the SERC CASE Award No. BC/117 (1985).

References

[1] Broadley KJ, Sykes SC, Davies RH. Towards a thermodynamic definition in partial agonism. Biochem Pharmacol 2010. Part I.
[2] Black JW, Leff P. Operational models of pharmacological agonism. Proc R Soc Lond B 1983;222:141–62.
[3] Weiland GA, Minneman KP, Molinoff PB. Fundamental difference between the molecular interactions of agonists and antagonists with the beta-adrenergic receptor. Nature 1979;281:114–7.
[4] Weiland GA, Minneman KP, Molinoff PB. Thermodynamics of agonist and antagonist interactions with mammalian beta-adrenergic receptors. Mol Pharmacol 1980;18:341–7.
[5] Contreras ML, Wolfe BB, Molinoff PB. Thermodynamic properties of agonist interactions with the beta-adrenergic receptor-coupled adenylate cyclase system. I: high and low affinity states of agonist binding to membrane-bound beta-adrenergic receptors. J Pharm Exp Ther 1986;237:154–64.
[6] Contreras ML, Wolfe BB, Molinoff PB. Thermodynamic properties of agonist interactions with the beta-adrenergic receptor-coupled adenylate cyclase sytem. II: agonist binding to soluble beta-adrenergic receptors. J Pharm Exp Ther 1986;237:165–72.
[7] Davies RH, Drug. Receptors in molecular biology. Int J Quant Chem Quant Biol Symp 1987;14:221–43.
[8] Broadley KJ, Sykes SC, Davies RH. The separation of agonist and antagonist components in the gross binding of a partial agonist on the cardiac (β-adrenoceptor and associated conformer thermodynamics. In: Szabadi E, Bradshaw CM, editors. Adrenoceptors: structure, mechanisms, function, Adv Pharm Sci Manchester 1991. Basel: Birkhauser; 1991. p. 375–6.
[9] Palczewski K, Kumaska T, Hori T, Behnke CA, Motoshima H, Fax BA, et al. Crystal structure of rhodopsin: AG protein-coupled receptor. Science 2000;289:739–45.
[10] Allen BPC, Nederkoorn PHJ, Timmerman H, Timms D, Broadley KJ, Davies RH. Monocation-driven proton transfer relays within G protein-coupled receptors of the rhodopsin class and the GTP synthase mechanism. J Mol Struct THEOCHEM 2008;859:51–68.
[11] Rasmussen SGF, Choi HJ, Rosenbaum DM, Kobilka TS, Thian FS, Edwards PC, et al. Crystal structure of the β_2-adrenergic G protein-coupled receptor. Nature 2007;450:383–7.
[12] Davies RH. A steady-state model of drug–receptor interaction *in vivo* applied to antagonists of the β-adrenergic receptor. Int J Quant Chem Quant Biol Symp 1977;4:413–42.
[13] Abraham MH. Free energies enthalpies and entropies of gaseous nonpolar nonelectrolytes in water and nonaqueous solvents. The hydrophobic effect. J Am Chem Soc 1982;104:2085–94.
[14] Cahill K, Parsegian VA. Rydberg-London potential for diatomic molecules and unbonded atom pairs. J Chem Phys 2004;121:10839–42.
[15] Davies RH, Bagnall RD, Jones WGM. A quantitative interpretation of phase effects in anaesthesia. Int J Quant Chem Quant Biol Symp 1974;1:201–12.
[16] Davies RH, Bagnall RD, Crooks B, Jones WGM. Anaesthetic potency and the perturbation of positive ion or proton acceptor sites in the nerve membrane. Int J Quant Chem Quant Biol Symp 1986;13:53–70.
[17] Nishikawa M, Kanno M, Kuriki H, Sugihara H, Motohashi M, Itoh K, et al. Life Sci 1975;16:305–14.
[18] Wilkinson AJ, Timms D, Davies RH, Broadley KJ, Kelly DR. Distortion and energetics in the agonist conformation of bound phenoxypropanolamine agents in the β_1-adrenoceptor. Int J Quant Chem Quant Biol Symp 1994;21:133–56.
[19] Wilkinson AJ, Davies RH. Vibrational modes in the antagonist and agonist action of ligands on the (1-adrenoceptor and some receptor site geometry. Int J Quant Chem Quant Biol Symp 1991;18:151–64.
[20] Broadley KJ, Nederkoorn PHJ, Timmerman H, Timms D, Davies RH. The ligand–receptor G protein ternary complex as a GTP synthase. Steady state proton pumping and dose–response relationships for β-adrenoceptors. J Theor Biol 2000;205:297–320.
[21] Weiss JM, Morgan PH, Lutz MW, Kenakin TP. The cubic ternary complex receptor-occupancy model. 1. Model description. J Theor Biol 1996;78:51–167.
[22] Weiss JM, Morgan PH, Lutz MW, Kenakin TP. The cubic ternary complex receptor-occupancy model. J Theor Biol 1996;178:169–82.
[23] Minneman KP, Hegstrand LR, Molinoff PB. The pharmacological specificity of beta-1 and beta-2 adrenergic receptors in rat heart and rat lung '*in vitro*'. Mol Pharmacol 1979;16:21–33.
[24] Buckner CK, Torphy T, Costa DJ. Studies on β-adrenoceptors mediating changes in mechanical events and adenosine 3′,5′-monophosphate levels, rat atria. Eur J Pharmacol 1978;47:259–71.
[25] Buckner CK, Wong SK. Stereoselectivity of isoproterenol-induced changes in cyclic AMP and mechanical events in rat atria and guinea-pig trachea. In: Szabadi E, editor. Recent advances in pharmacology of receptors. Amsterdam: Elsevier BV; 1978. p. 319–20.
[26] Birnbaum JE, Abel PW, Amidon G, Buckner CK. Changes in mechanical events and adenosine 3′,5′-monophosphate levels induced by enantiomers of isoproterenol in isolated rat atria and uteri. J Pharm Exp Ther 1975;194:396–409.
[27] Broadley KJ. Cardiac β-adrenoceptors. J Auton Pharmacol 1982;2:119–45.
[28] Kaumann AJ, Birnbaumer L. Desensitisation of kitten atria to chronotropic, inotropic, and adenylyl cyclase stimulating effects of (−) isoprenaline. Naun-Schmied Arch Pharm 1976;293:199–202.
[29] Broadley KJ, Nicholson CD. Functional antagonism as a means of determining dissociation constants and relative efficacies of sympathomimetic amines in guinea-pig isolated atria. Br J Pharmacol 1979;66:397–404.
[30] Kaumann AJ. In kitten ventricular myocardium, the inotropic potency of an agonist is determined by both the intrinsic activity for the adenylyl cyclase and its affinity for the β-adrenoceptors. Naun-Schmied Arch Pharm 1981;317:13–8.
[31] Sakmann B, Noma A, Trautwein W. Acetylcholine activation of single muscarinic potassium ion-channels in isolated pacemaker cells of the mammalian heart. Nature 1983;303:250–3.
[32] Soejima M, Noma A. Mode of regulation of the Ach-sensitive K-channel by the muscarinic receptor in rabbit atrial cells. Pflueger's Arch 1984;400:424–31.
[33] Horstmann DA, Brandon S, Wilson LA, Guyer Jr EJ, Limbird LE. An aspartate conserved among G protein receptors confers allosteric regulation of α-2adrenergic receptors by sodium. J Biol Chem 1990;265:21590–5.
[34] Neve KA, Cox BA, Henningsen RA, Spannoyannis A, Neve R. Pivotal role for aspartate-80 in the regulation of dopamine D2-receptor affinity for drugs and inhibition of adenylyl cyclase. Mol Pharmacol 1991;39:733–9.
[35] Strader CD, Sigal IS, Candelore MR, Rands E, Hill WS, Dixon RAF. Conserved aspartic acid residues 79 and 113 of the β-adrenergic receptor have different roles in receptor function. J Biol Chem 1988;263:10267–71.
[36] Brooks BR, Bruccoleri RE, Olafson BD, States DJ, Swaminathan M, Karplus J. CHARMM: a programme for macromolecular energy minimisation and dynamics calculations. J Comp Chem 1983;4:187–217.