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COMMENTARY

ON THE FUNDAMENTAL DIFFERENCE IN THE THERMODYNAMICS OF AGONIST AND ANTAGONIST INTERACTIONS WITH β-ADRENERGIC RECEPTORS AND THE MECHANISM OF ENTROPY-DRIVEN BINDING

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Several investigations point to a fundamental difference between the molecular interactions of agonists and antagonists with the β-adrenergic receptor. Whereas β-agonists bind with large decreases in enthalpy and entropy, the binding of β-antagonists is characterized by a small change of enthalpy and a large increase in entropy. Moreover, the difference in binding is reflected in the temperature dependence of the affinities. Agonists display large increases in affinity with temperature decreases, whereas the affinities of antagonists change little. The negative entropy change in agonist binding is usually ascribed to a supposed conformational change of the receptor. The increase of entropy in antagonist binding is assumed to be due to a hydrophobic binding mechanism. In this commentary, after a review of the experimental results, we critically assess the current interpretation, giving reasons as to why it may not be correct, and propose an alternative interpretation of the thermodynamic data in question, including the temperature dependence.

Experimental findings

In a well-known paper [1], Weiland *et al.* reported observing fundamental differences between molecular interactions of agonists and antagonists with the β-adrenergic receptor of turkey erythrocytes (Table 1). Antagonist binding was found to be largely entropy driven, with only a small enthalpy component. The binding of agonists, on the other hand, was associated with a large decrease in enthalpy and a highly unfavourable decrease in entropy. Furthermore, full agonists displayed large increases in affinity with lowering of temperature whereas the affinities of antagonists were essentially unchanged. The observation that agonist, but not antagonist, affinities for β-adrenergic receptors increase at lower temperature is confirmed also by the work of other authors [2–10]. The thermodynamics of ligand binding to β-adrenergic receptors in mammalian tissues has also been studied [11]. Since two β-receptor subtypes (β_1 and β_2) exist in such tissues, four cases have been considered: the tissues containing mostly β_1-receptor (cerebral cortex, heart), mostly β_2-receptor (cerebellum, lung), and the tissues in which the effects of GTP on agonist affinity for the receptor are observed (cerebellum, heart, lung), or are not observed (cortex). The antagonist binding was, in all cases, characterized by favourable entropy increases, in marked contrast to the unfavourable entropy decreases associated with the binding of agonists. GTP had no effect on antagonist binding at any temperature examined ($T = 50°$ and 25°). The affinities of antagonists for receptors in mammalian tissues were relatively insensitive to changes in temperature. The affinities of agonists increased when temperature was decreased in all cases considered. In the presence of GTP, the agonist affinities were less sensitive to temperature in cerebellum, heart and lung (where GTP effects are observed), and the changes in enthalpy and entropy were less negative. The above results suggest that the thermodynamic difference between agonist and antagonist binding may be a general characteristic of β-adrenergic receptors. Further support for this hypothesis was provided by the thermodynamic properties of binding to membrane-bound as well as to solubilized β-adrenergic receptors [12, 13]. According to the present understanding, the antagonists are believed to passively occupy the receptor and thus block the interaction between agonists and receptors. On the other hand, the agonists (H) are thought to first bind to the receptors (R) to form a low-affinity complex H·R which subsequently interacts with the guanine nucleotide-binding protein N_s, thus forming a high-affinity ternary complex H·R·N_s [14]. Formation of H·R·N_s facilitates the exchange of GTP for GDP on N_s, and binding of GTP to N_s in the presence of divalent cations leads to the destabilization of the ternary complex. The activated N_s is then believed to activate the catalytic moiety. In the presence of GTP, H·R·N_s does not accumulate and binding reflects the formation of the H·R complexes. The binding of antagonists is thought not to involve an interaction with N_s. Consistent with the last two assertions are the observations that binding of agonists to soluble receptors is associated with thermodynamic parameters similar to those for binding of agonists to membrane-bound receptors in the presence of GTP and that the thermodynamic data for antagonists are quantitatively similar in the soluble and in the membrane-bound case [12, 13]. The interaction of antagonists was entropy driven and was rather insensitive to temperature in all cases. The

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Table 1. Thermodynamic parameters of ligand binding to the β-adrenergic receptor of turkey erythrocytes and the ratios of the corresponding equilibrium dissociation constants K_D at 1° and 37° [1]

	$\Delta G°$ (kcal/mol)	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal/mol deg)	$\frac{K_D\ (37°)}{K_D\ (1°)}$
Agonists				
(−)Isoproterenol	−9.39	−13.39	−12.9	23.3
(−)Norepinephrine	−7.91	−18.86	−35.3	55.5
(−)Epinephrine	−7.50	−12.75	−16.9	16.0
Partial agonists				
Soterenol	−8.23	−7.84	+1.26	5.3
Fenoterol	−7.81	−6.06	+5.65	3.6
Salmefamol	−7.28	−8.86	−5.10	6.6
Metaproterenol	−6.78	−10.83	−13.06	10.1
Terbutaline	−6.19	−4.13	+6.65	2.4
Antagonists				
(−)Propranolol	−12.51	−3.85	+27.9	2.6
MK-950	−12.32	+0.81	+42.3	0.84
IPS-339	−12.30	+0.25	+40.5	0.95
Pindolol	−11.85	−5.08	+21.8	2.95
Zinterol	−9.13	−3.06	+19.6	1.92
Metoprolol	−8.36	−0.66	+24.8	1.15
Sotalol	−8.21	−2.15	+19.5	1.58
H35/25	−7.96	−1.83	+19.8	1.48
Butoxamine	−7.71	−1.08	+21.4	1.26
OPC 2009	−7.55	−0.90	+21.5	1.21
Atenolol	−7.49	−3.47	+13.0	2.09
Practolol	−7.46	+3.92	+36.7	0.45

interaction of the receptor with the regulatory protein N_s to form the high-affinity state of agonist binding was thermodynamically characterized by larger negative changes in enthalpy and entropy than formation of the low-affinity state of agonist binding [12, 13]. The high-affinity dissociation constant, K_H, decreased much more with decreasing temperature than the low-affinity dissociation constant, K_L. Binding of partial agonists showed a temperature sensitivity generally intermediate between that of full agonists and the antagonists propranolol and timolol, with the high-affinity binding states being more sensitive to temperature than the low-affinity ones.

It is impossible, however, to generalize from one hormone or neurotransmitter system to another with respect to specific thermodynamic changes associated with ligand–receptor interactions. Striking differences have been found, e.g. between the thermodynamic parameters associated with ligand–receptor interactions in the β-adrenergic system and the dopaminergic system [15–17]. In the latter case, both agonist and antagonist binding may be entropy or enthalpy driven.

Difficulties with the current interpretation

In discussing the above results, we first note that if binding of a ligand is tight (as in a covalent bond), six additional vibrational modes appear in the system. The loss of the translational and rotational entropy is typically ~35 e.u. (cal per mol per degree) whereas the entropy of a vibrational mode is ~1 e.u. [18]. The six extra vibrations appearing with binding thus cannot compensate for the loss of the translational and rotational entropy. If the bonds are weaker, as for example in hydrogen bonded and charge transfer complexes, the observed entropies of complex formation are still on the order of −10 to −20 e.u. [18]. The entropy loss is smaller in this case, mainly because of internal rotations and various low-frequency motions. The negative entropy change observed in binding of agonists to β-receptor may thus be regarded as "normal," since the bonds are rather weak. A more complete discussion of this point will be presented elsewhere; here we only want to stress that the induced conformational change suggested in the original work [1] (and in a number of subsequent papers) as a possible cause of the negative entropy change in agonist binding is not needed to rationalize the data. It can be, of course, that such a conformational change does occur; the thermodynamic data, however, do not provide support for such an assumption, nor do they exclude it. The conformational change triggered by agonist binding may primarily concern the receptor segment involved in the interaction with the N_s protein. It may mainly consist in keeping certain binding groups of the receptor at the proper separation, so that the N_s protein can bind. This type of mechanism (which seems to be present, e.g. in the *trp* repressor/operator system [19]) would entail stiffening of certain bonds which should lead to a negative change of entropy that is small compared with the rotational and translational entropy loss [18].

The most striking aspect of the thermodyamic data described above certainly lies in the positive change of entropy which has been observed persistently in binding of antagonists to the β-adrenergic receptor. Binding processes with a positive change of entropy (the entropy-driven processes) are often observed in

biological systems. They play a fundamental role, e.g. in polymerization of numerous proteins, in antigen–antibody reactions, and in the actin-myosin reaction (the principal reaction in muscle contraction). So far it has been usually assumed that the positive change of entropy in binding was due to hydrophobic interactions. The hydrophobic origin, therefore was also adopted in rationalizing the observed positive entropy change in β-antagonist binding. Although the hydrophobic effects undoubtedly play a major role in some of the processes mentioned, there are, as we shall show below, good grounds to believe that they may not be the source of the positive entropy change in the case in question.

The thermodynamic analysis was based on studies of the standard free energy change, ΔG°, in the association reactions. From the equations of equilibrium thermodynamics

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \qquad (1)$$

and

$$\Delta G^\circ = -RT \ln K_A \qquad (2)$$

where K_A is the equilibrium association constant, one obtains the van't Hoff equation

$$\ln K_A = -\Delta H^\circ/RT + \Delta S^\circ/R. \qquad (3)$$

Most important to note is that plots of $\ln K_A$ vs $1/T$ were linear (in the temperature range 278–310°K) for both β-agonists and antagonists [1, 11–13, 20, 21], showing that the enthalpy as well as the entropy changes were independent of temperature. From the slope of the plot, ΔH° can thus be obtained, and then ΔS° can be calculated via Equations 1 and 2.

The binding of β-antagonists is largely entropy driven, the corresponding change of enthalpy being rather small. It is difficult, however, to reconcile the observed linearity of the van't Hoff plots with the (generally) supposed hydrophobic origin of the binding mechanism for antagonists. The positive ΔS° in this case, would be due to the decrease of the local order in water molecules adsorbed on the ligand surface when transferred back to the bulk water during the binding. One would expect this kind of molecular rearrangement to be rather strongly temperature dependent, and it is indeed found [22–24] that the corresponding van't Hoff plots are curved and that the enthalpy and entropy changes are strongly temperature dependent. In Fig. 1, two examples are given of the temperature variation of the ΔH° and ΔS° due to the hydrophobic effect. The linearity of the van't Hoff plots is thus not in accord with the hydrophobic binding mechanism. (When the present work was in preparation, we found out that this had been already pointed out by another group of authors [20].) Further evidence in support of our conjecture, that there are other mechanisms of positive entropy binding besides the hydrophobic interaction, is provided by the thermodynamic analysis of the interaction of antagonists with the muscarinic acetylcholine receptor [25]. The situation is illustrated in Fig. 2. Whereas the van't Hoff plots of β-antagonists and agonists are all linear (we give pindolol as an example), the corresponding plots of the muscarinic antagonists atropine and guinuclidinyl

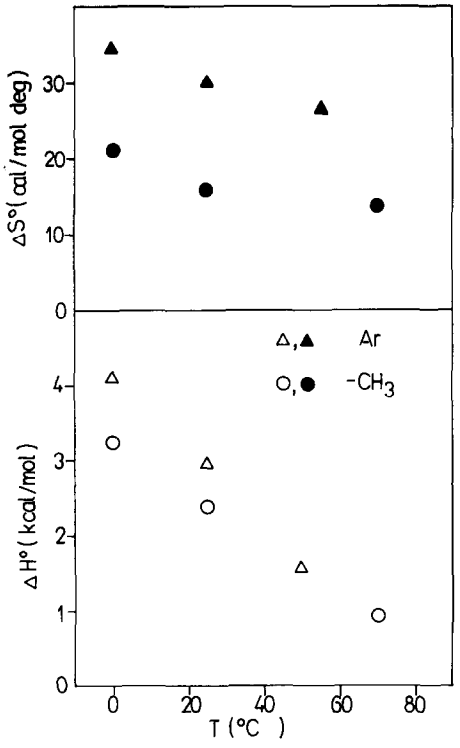

Fig. 1. Temperature dependence of ΔH° and ΔS° for the transfer from aqueous solution (unit mole fraction) to gas (1 atm). The results are given per methyl group of alkanes (○, ●) and for argon (△, ▲) [24].

benzilate (QNB) differ qualitatively from each other. Both molecules bind to muscarinic acetylcholine receptors with a positive change of entropy. The curved plot of QNB reflects the decreasing of ΔS° and ΔH° when temperature is increased. One would indeed expect such behaviour if binding is dominated by hydrophobic interaction [23, 24]. The linear van't Hoff plot of atropine shows that the entropy and enthalpy changes are essentially independent of temperature in this case. The two kinds of temperature dependence of the binding constant can be regarded as a strong indication that at least two distinct mechanisms may exist in the case of the entropy-driven binding in question.

The hydrophobic character of β-antagonist binding may be questioned further on the grounds of the recent studies [21] of ΔG°, ΔH° and ΔS° dependence on lipophilic properties (which were determined by reverse-phase high performance liquid chromatography). Twenty compounds were investigated, sixteen antagonists and four agonists (one partial). ΔH° and ΔS° turned out to correlate poorly with lipophilicity ($r \sim 0.6$), and the correlation of ΔG° was even worse ($r \sim 0.4$). These results are not in agreement with the apparently quite general property of hydrophobic interactions that ΔG° correlates well with lipophilicity but that the corresponding changes in enthalpy and entropy show no such regularity [22].

On completing the work on our model we became aware of even more direct evidence against the

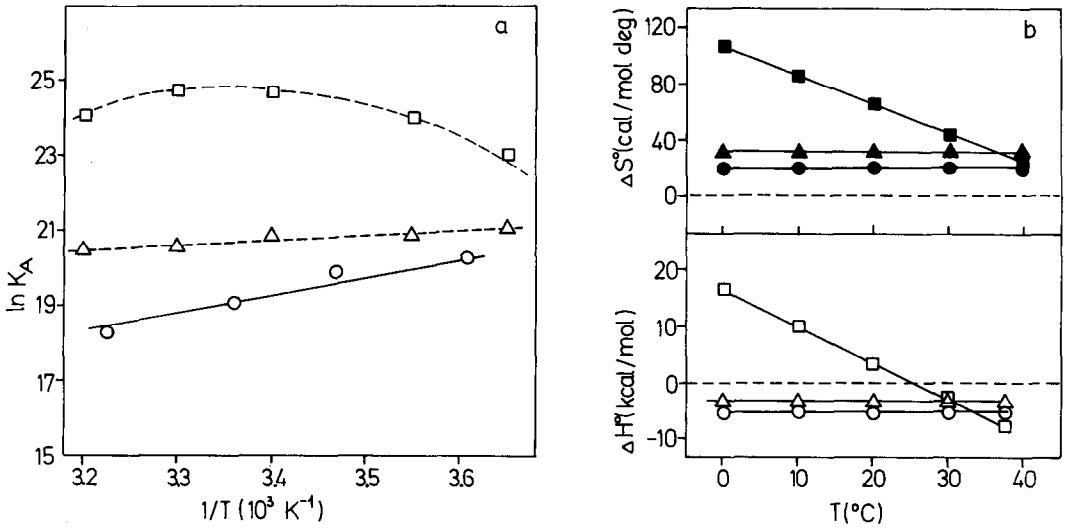

Fig. 2. (a) van't Hoff plots of the binding of the antagonist pindolol (○) to β-adrenergic receptors in lung membranes [21] and of the binding of the antagonists atropine (△) and QNB (□) to the muscarinic acetylcholine receptor in heart membranes [25]. (b) Temperature dependence of $\Delta H°$ and $\Delta S°$ in the binding of pindolol (○, ●), atropine (△, ▲) and QNB (□, ■).

hydrophobic binding mechanism of β-adrenergic ligands. A number of investigations [26–30] have been reported recently which indicate that β-adrenergic ligands interact with the receptor in a manner topologically analogous to that of retinal interacting with opsin, although the nature of the binding forces is different. More specifically, this means that the binding site for the β-adrenergic ligand is in a hydrophobic region of the receptor formed by a bundle of seven transmembrane segments. Direct participation of water molecules in the binding process thus seems to be excluded.

Proposed alternative interpretation of the entropy increase in binding of β-adrenergic antagonists

The facts put forward above strongly indicate that the hydrophobic interaction cannot provide the mechanism for the entropy-driven binding of β-adrenergic antagonists (or for binding of the muscarinic antagonist atropine). Indeed, they constitute compelling evidence against the general validity of the usual assumption that entropy-driven binding is of hydrophobic origin.

In an attempt to obtain a consistent interpretation of the thermodynamic data on binding of β-adrenergic ligands (including the temperature dependence of the affinities), we note first that, together with the entropy increase, small changes of enthalpy (Table 1) which are either positive or negative can also be regarded as a characteristic of β-antagonist binding. Whereas the large decrease of enthalpy and entropy in the case of agonists indicates binding in a tighter complex in which translational and rotational degrees of freedom of the ligand are lost (six vibrations appear instead), the small enthalpy changes of antagonists seem to be consistent with formation of a "loose complex." In such a complex, translational and rotational degrees of freedom may be preserved, at least partially, although they may be strongly coupled so that the ligand undergoes "jump reorientational motion," similar as studied recently (in other systems) in 2D NMR experiments [31]. We have proposed recently [32] that positive entropy change in β-antagonist binding may be due to the transition from a weak to a strong translation–rotation coupling regime upon formation of a loose complex. Although experimental evidence exists for such a mechanism in other systems, we now feel that it is not plausible in the case in question, considering the present picture of β-ligand binding [26–30]. According to the present understanding, the ligand may be regarded as being dissolved in the transmembrane region of the protein composed of seven helices. It thus moves in a hydrophobic channel, the cross section of which varies because of the distortions and dislocations in some of the helical elements [33]. The motion of the ligand in the channel may be expected to be largely restricted, rotationally and even translationally, so that it can be regarded as essentially one dimensional. On reaching the binding cavity such a ligand, if an agonist, may bind tightly, connecting two of the helices [26–30]. It thus looses the entropy which it had in the restricted motion in the free state. The observed negative $\Delta H°$ and $\Delta S°$ changes are consistent with this assumption. If the ligand is an antagonist, it may only form a loose complex in the cavity (with $\Delta H°$ small) where its translational and rotational motion may be less restricted than in the unbound state in the channel. The binding of antagonists is thus entropy driven because the number of the available states of an antagonist increases upon the transition from the free state in the narrow part of the channel to the loose bound state in the cavity. This situation is schematically depicted in Fig. 3.

The binding modes suggested above to explain qualitatively the fundamental difference in the $\Delta H°$ and $\Delta S°$ values between β-agonist and antagonist

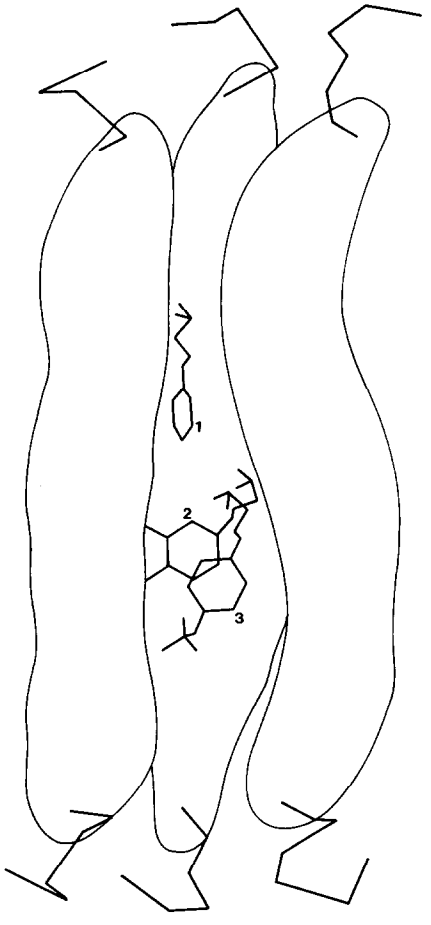

Fig. 3. A β-ligand (1) moving from the narrow part of a transmembrane channel into the binding cavity forms either a tight complex (2), if an agonist, or a loose complex (3), if pure antagonist. In the loose complex the ligand has more motional freedom than in the channel which leads to the entropy increase in complex formation. In the case of partial agonists, some of the ligand molecules may bind tightly (like agonists) and some loosely (like pure antagonists). The ratio of the two types of complexes (and thus the intrinsic activity) should depend on the details of the interaction sites and on temperature.

binding are also consistent with the experimental temperature dependence of the respective dissociation constant $K_D = 1/K_A$. The values of K_D (37°)/K_D (1°) given in Table 1 can be calculated from Equation 3 provided that we suppose $\Delta H°$ and $\Delta S°$ to be nearly temperature independent. As we argued, this assumption is in accord with the linear van't Hoff plots, but not with the hydrophobic binding mechanism. If we assume for simplicity that the ligand has no rotational degrees of freedom when moving in the channel, then the entropy change of an agonist upon tight binding in the cavity equals approximately the loss of its translational entropy and is therefore negative

$$\Delta S_{ag} \simeq \Delta S_{tr} = -k \ln \left[\left(\frac{2\pi MkT}{h^2}\right)^{3/2} e^{5/2}\, V_{ch}\right] \quad (4)$$

where M is the mass of the agonist and V_{ch} is the volume available in the free state. We assume only one ligand molecule in the channel and neglect the complications due to the fact that there are N ligand molecules in the system. (For the point we are trying to make here, this is not essential.) Antagonists, on the other hand, may gain rotational degrees of freedom upon transition from the channel to the binding cavity, because the bound state they form there may be very loose. Provided that just one rotational degree of freedom is gained, the entropy change ΔS_{antg} of an antagonist is then:

$$\Delta S_{antg} = \Delta S_{tr} + \Delta S_r$$
$$= k \ln (V_c/V_{ch}) + k \ln (8\pi^2 eI\, kT/h^2) \quad (5)$$

where V_c is the volume the ligand occupies in the cavity and I is its moment of inertia about the axis of allowed rotation. Symmetry number 1 is assumed. The gain in the rotational entropy may outweigh the loss of the translational entropy in Equation 5 so that ΔS_{antg} can be positive. The entropy change depends on the temperature only logarithmically, which amounts to a variation of only about 2% in the relevant temperature interval from 1° to 37°. Our model thus correctly reproduces the major features of the entropy and enthalpy behaviour of agonist and antagonist binding to the β-adrenergic receptor.

The question remains whether there are any structural characteristics of β-ligands supporting our model. To discuss this point, let us consider the structure of the agonist isoproterenol, the partial agonist dichloroisoproterenol (DCI), and the antagonist sotalol which shows no agonist activity [34] (Fig. 4). All three of them have identical side chains and differ only in the aromatic moiety. The 3,4-OH groups of isoproterenol form a pronounced binding site characterized by a region of fairly strong negative electrostatic potential. Considering also the side chain sites, we may suppose that isoproterenol binds in a multiple-site bond, thus forming a fairly tight complex. Relatively large negative $\Delta H°$ and $\Delta S°$ values support this assumption and so does, for example, the correlation between the affinity and the electrostatic potentials of phenylethylamine derivatives [35] (which in fact can also be regarded as evidence that the proposed binding mode of β-agonists is general). The antagonist sotalol does not have a comparable region of the negative potential (see also Ref. 36), and it may be assumed that bond formation does not take place near the 3,4 sites. Sotalol is loosely bound (Table 1), apparently through the side chain binding sites, and it may be assumed to undergo large amplitude "jump reorientational motion" in the binding cavity. Its entropy thus increases upon transition from the narrow channel to the cavity. DCI is an antagonist, with partial agonism so strong that it cannot be used as a drug [37]. DCI does have a region of negative potential near the 3,4 sites, but it is considerably weaker than that of isoproterenol. Basic statistical theory leads us then to assume that some of the DCI molecules bind at 3,4 sites, thus forming a tighter complex which has an agonistic effect, and some do not and these are loosely bound and act as antagonists. Their

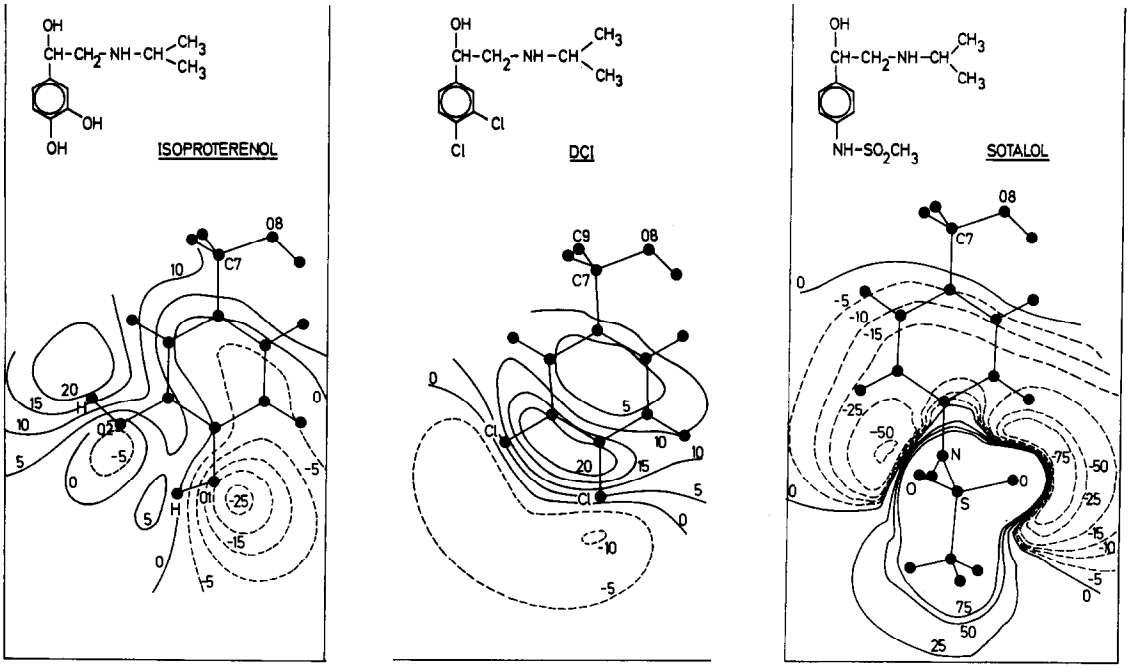

Fig. 4. Electrostatic potential maps of the β-agonist isoproterenol, the partial agonist DCI, and the pure antagonist sotalol calculated at 1.7 Å above the aromatic ring plane (*ab initio* calculations with STO-3G basis). Dashed and full equipotential lines represent negative and positive potential respectively. Units are 0.627 kcal/mol.

equilibrium fraction and thus the intrinsic activity should primarily depend on the interaction strength at the relevant binding sites and on the temperature. This picture may offer a general explanation of partial β-agonism and may be applicable in other cases as well. It is supported by the thermodynamic values of partial agonists laying between those of agonists and pure antagonists [1, 11–13]. Our hypothesis seems to be in accord with the findings of a more extensive recent study [36] of molecular electrostatic potentials of partial β-agonists and pure antagonists. It may also provide an explanation for some of the other puzzling properties of partial β-agonists [38–43], particularly of the biphasic dissociation [41, 43]. We will study these properties in subsequent papers.

After having completed the paper, a very recent review article [44] on thermodynamic analysis of the drug–receptor interaction came to our attention. One of the main conclusions there, that the temperature dependency of the dissociation constant is a property of the drug–receptor interaction that appears to be as characteristic and as informative as the dissociation constant itself, is fully in agreement with the spirit of our work. Hopefully we added further weight to this observation and also provided some insight.

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