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COMMENTARY

DO THERMODYNAMIC STUDIES PROVIDE INFORMATION ON BOTH THE BINDING TO AND THE ACTIVATION OF DOPAMINERGIC AND OTHER RECEPTORS?

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Many studies [e.g. Refs. 1–4] have shown that the binding of ligands to their receptors is a temperature-dependent process. Although this dependence has often been described, only in recent years have thermodynamic parameters been used to gain more information on drug–receptor interactions. As detailed later, binding may be separated into an enthalpic and an entropic component, each of which contains related but different information.

Weiland *et al.*, when investigating the binding of agonists and antagonists to adrenoceptors, made the fundamental observation that the binding of agonists was enthalpy-driven whereas that of antagonists was entropy-driven [5, 6]. They interpreted the enthalpy-driven agonist–receptor binding to represent a conformational change in the receptor leading to the biological response. In contrast, entropy-driven binding has been suggested to correspond to an interaction without transfer of information. Comparable changes were seen with the benzodiazepine receptor, where the binding of the agonist diazepam was enthalpy-driven while that of the antagonist Ro 15-1788 was both enthalpy- and entropy-driven [7]. However, not all neurotransmitter systems exhibit similar thermodynamic behaviour upon agonist and antagonist interaction. For example, Hitzemann *et al.*, when examining the opiate receptor for an agonist-induced conformational change (postulated to be an enthalpy-driven process), found that the binding of the antagonist diprenorphone was enthalpy-driven whereas that of the agonist etorphine was entropy-driven [8]. Similarly, Maelicke *et al.* [9] found that agonist binding to the nicotinic receptor was entropy-driven.

From the above papers, we note that agonists and antagonists appear to consistently behave in opposed fashion (enthalpy- versus entropy-driven binding), suggesting that thermodynamic studies indeed allow a pharmacological mode of action to be recognised at the molecular level. However, we also note that, depending on the type of receptor being examined, either mode of binding can characterise agonists or antagonists. This is certainly not easy to reconcile with the hypothesis that the transfer of information following receptor activation by an agonist should determine the thermodynamic characteristics of the binding process. A critical observation at this stage is that all relevant studies available suffer from the same shortcoming, namely that the compounds investigated are rather limited in number and have comparable physicochemical properties within each group. We thus felt that, before pursuing further rationalisations, it would be essential using a large and diversified series of ligands to examine: (a) whether agonists and antagonists necessarily bind by distinct thermodynamic modes, and (b) what kind of information, physicochemical and/or functional, can be extracted from thermodynamic binding parameters.

Such a study was undertaken using four agonists and fifteen antagonists of the dopamine D-2 receptor [10]. The present commentary mainly focuses on the results of this study and on their mechanistic interpretation, suggesting that, at least for the D-2 receptor, thermodynamic binding parameters are determined by structural features more than by the pharmacological properties of the ligands.

THERMODYNAMIC ANALYSIS OF RECEPTOR BINDING

Theoretical and experimental aspects

Thermodynamic analysis represents a method of determining the underlying driving forces of binding. The free energy of binding (standard free energy change ΔG°) can be resolved into enthalpic and entropic components, defined thus:

—Enthalpy, the heat content of a substance per unit mass;
—Entropy, a measure of energy existing but unavailable for work, i.e. a measure of the state of disorder of the system.

Changes in the standard free energy are related to changes in enthalpy (ΔH° in kcal·mol^{-1}) and in entropy (ΔS° in cal·mol^{-1}·K^{-1}) by the Gibbs

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equation:

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

where T is the absolute temperature in Kelvin.

Experimentally, one proceeds by measuring K_i (the equilibrium dissociation constant between a drug and a receptor) at different temperatures and calculating the corresponding free energy changes:

$$\Delta G^\circ = -RT \cdot \ln 1/K_i \quad (2)$$

where R is the gas constant ($1.987\ \text{cal} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$). When the change in enthalpy is constant over the temperature range examined, ΔH° can be obtained from the slope of Van't Hoff plots (i.e. plots of $\ln 1/K_i$ versus $1/T$) by using a combination of equations 1 and 2:

$$\ln 1/K_i = (-\Delta H^\circ/R) \cdot 1/T + \Delta S^\circ/R \quad (3)$$

It is observed often [11, 12] that changes in enthalpy are correlated linearly with changes in entropy. The phenomenon is termed enthalpy–entropy compensation, and it exists as long as the following relationship is verified:

$$\Delta G^\circ = \Delta H^\circ \cdot (1 - T/\beta) \quad (4)$$

The existence of enthalpy–entropy compensation has been shown to imply that a single mechanism predominates in the process investigated [13]. The proportionality factor β between ΔH° and ΔS° has the dimension of an absolute temperature and is called the isokinetic temperature, i.e. the temperature at which all reactions in the series proceed at the same rate. The macroscopic meaning of this temperature is not clear, but it may have significance at the molecular level. Enthalpy–entropy compensations thus appear as a promising new development in thermodynamic studies.

A molecular view

A simplified diagram of the likely entropy (ΔS°) and enthalpy (ΔH°) changes occurring upon binding of a ligand to a receptor is presented in Fig. 1. The following thermodynamic events can be recognised upon ligand binding:

(a) Complex formation *per se* implies increased orderliness and hence an energetically unfavourable entropy decrease ($-\Delta S^\circ$).
(b) Hydrophobic bonds between complementary apolar regions of both partners are mainly entropic in origin, i.e. they are entropy-driven ($+\Delta S^\circ$).
(c) The various electrostatic bonds (ion—ion, ion—dipole, dipole—dipole interactions, hydrogen bonds) between complementary polar groups are driven by a comparatively strong decrease in enthalpy ($-\Delta H^\circ$). In addition, the concomitant dehydration of the binding groups increases the disorder of the system, i.e. an entropy drive also exists ($+\Delta S^\circ$).

It is a generally accepted view that agonists act by inducing in the receptor conformational or other changes that result in its activation and that trigger the cascade of events leading to the pharmacological response. In contrast, the molecular action of antagonists is a receptor blockade preventing the binding of agonists. From a thermodynamic viewpoint as schematised in Fig. 1, no changes should occur following the formation of a receptor–antagonist complex. In contrast, receptor activation following agonist binding implies changes in enthalpy and entropy, although we believe the direction of these changes to be a matter of speculation at present (Fig. 1). From a study of agonist binding to β-adrenoceptors, Contreras *et al.* [14, 15] concluded that complex formation is entropy-driven, while the two subsequent steps of receptor activation and binding of the stimulatory component of adenylate cyclase are both enthalpy-driven ($-\Delta H^\circ$ overcompensating a small $-\Delta S^\circ$). However, one can just as easily imagine entropy-driven activation steps, assuming for example a marked dehydration.

Perhaps more important in this context is a delineation of the information afforded by thermo-

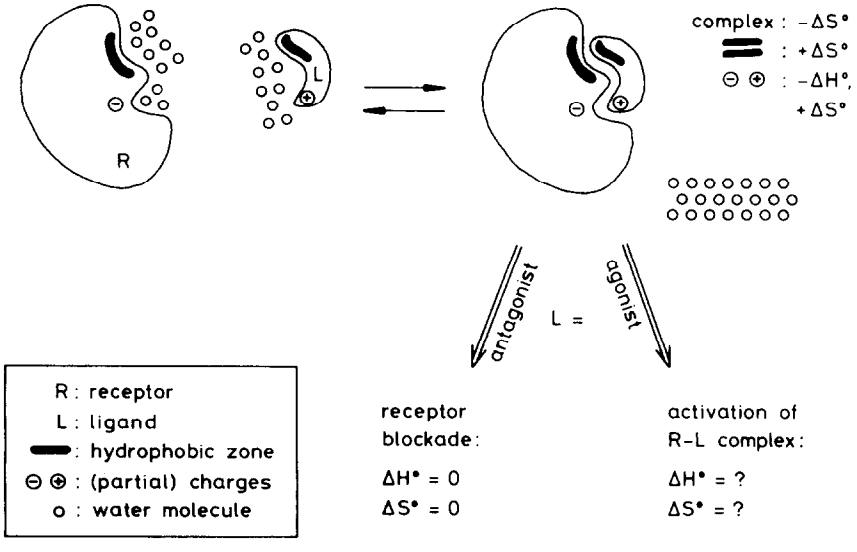

Fig. 1. Molecular and (postulated) thermodynamic events in the binding of drugs to receptors.

Table 1. Thermodynamic parameters of the displacement of [^{3}H]spiperone from rat striatal preparations, lipophilicity (log k_w), and apolar surface area (SA) of dopamine receptor ligands [10]

Compounds	$\Delta G°$* (kcal·mol^{-1})	$\Delta H°$ (kcal·mol^{-1})	$\Delta S°$* (cal·mol^{-1}·K^{-1})	log k_w† (pH 7.4)	Apolar SA (Å^2)
Agonists					
6,7-ADTN	−9.7	−16.4	−24.2	−0.42	127
Dopamine	−8.2	−8.7	−1.8	−0.81	108
Pergolide	−9.7	0.7	37.4	3.48	286
Apomorphine	−8.7	0.8	34.4	2.25	214
Antagonists					
Sulpiride	−11.3	−21.2	−35.7	0.61	240
Piquidone	−11.6	−17.4	−20.9	1.35	229
Tiapride	−6.7	−14.2	−27.0	−0.54	259
Metoclopramide	−10.3	−13.1	−10.3	1.05	254
Clebopride	−12.4	−12.8	−1.4	2.86	309
Alizapride	−10.4	−12.2	−6.6	2.52	245
(−)−Sultopride	−11.4	−12.2	−3.0	0.77	286
Zetidoline	−11.1	−11.5	−1.3	2.15	295
YM 09151-2	−12.3	2.9	54.8	3.52	339
Raclopride	−9.8	3.6	48.3	2.41	271
Haloperidol	−11.6	−3.0	31.0	3.11	341
Clozapine	−8.6	0.4	32.5	2.99	281
cis-Flupenthixol	−9.8	3.6	48.4	4.44	358
[^{3}H]Spiperone	−12.0	5.2	61.8	3.28	347
(+)−Butaclamol	−9.8	13.5	84.2	5.01	340

* Value at 4°.
† In the original publication [10], most of these values were rounded to the first decimal place.

dynamic binding studies. Displacing a labeled ligand of high affinity and selectivity is a means of determining the relative affinities of a number of other ligands, but to rationalise such binding data in terms of a subsequent receptor activation step appears as an overinterpretation in the absence of functional data. In their study quoted above, Contreras *et al.* [14] correlated thermodynamic binding data of agonists with their efficacy in activating adenylate cyclase, but statistical correlations do not imply causal relationships.

BINDING TO THE DOPAMINE D-2 RECEPTORS

A thermodynamic study

We have reported recently the thermodynamic behaviour of a large series of dopamine agonists and antagonists binding to the dopamine D-2 receptor [10]. In this study, the ligands' lipophilicity, one of the most important physicochemical properties of bioactive molecules, was assessed by the hydrophobic index (log k_w) determined by RP-HPLC [16]. The thermodynamic parameters given in Table 1 show that enthalpy- and entropy-driven binding occur for agonists and for antagonists alike. Among the agonists, dopamine and 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene hydrobromide (ADTN) were more potent in displacing [^{3}H]spiperone at lower temperature (4°), implying exothermic binding ($-\Delta H°$) which more than compensates for the unfavourable decrease in entropy. In contrast, the affinities of pergolide and apomorphine were relatively insensitive to temperature (4–37°) and hence their binding was entropy-driven.

Among the antagonists, the affinity of classical neuroleptics (e.g. haloperidol, flupenthixol and butaclamol) was either little affected by temperature or enhanced at 37°. Therefore, here again, the drugs display entropy-driven binding. Two lipophilic benzamides (YM 09151-2 and raclopride) behaved similarly. In contrast, the behaviour of the more hydrophilic antagonists, i.e. most substituted benzamides (sulpiride, tiapride, metoclopramide, etc.) as well as piquidone and zetidoline, is particularly intriguing. Not only is the receptor binding of these atypical neuroleptics enthalpy-driven, but there is also abundant reference in the literature that it is sodium-dependent. At this stage, we can only ask ourselves whether the two phenomena are connected at the molecular level.

In summary, our investigation showed that the thermodynamic driving forces of binding to D-2 receptors do not relate directly to the intrinsic activity of the drugs. Indeed, the binding of the relatively hydrophilic agonists and antagonists was enthalpy-driven, whereas lipophilic agonists and antagonists appear to share entropy-driven binding.

Relationships between physicochemical properties and entropy of binding

The above discussion suggests that for the data in Table 1 lipophilicity may be related to the enthalpy and entropy of binding. These two thermodynamic parameters being highly intercorrelated ($r = 0.99$) due to enthalpy–entropy compensation, only entropy

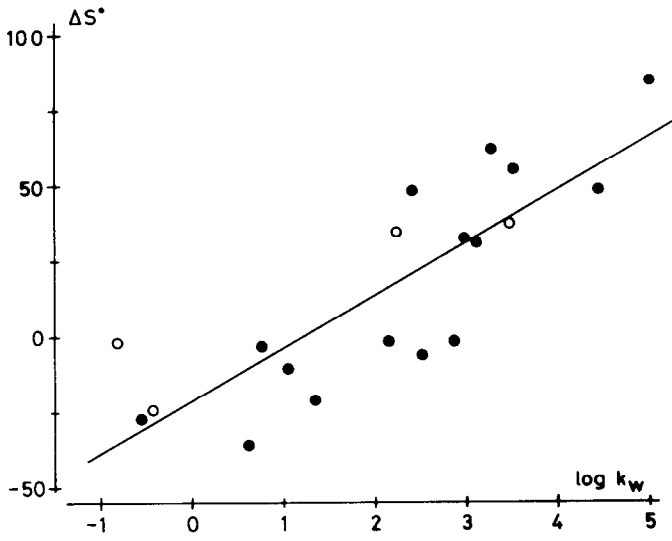

Fig. 2. Correlation ($r = 0.83$) between apparent drug lipophilicity (log k_w at pH 7.4) and observed entropy change ($\Delta S°$ at 4°) for the binding to the dopamine D-2 receptor [10]. Key: (○) agonists; and (●) antagonists.

is considered below. It was indeed found (Fig. 2) that lipophilicity and the change in entropy of binding are fairly well correlated ($r = 0.83$) such that, the more lipophilic the ligand, the more entropy-driven its binding. This indicates that the increase in entropy upon D-2 receptor binding should reflect an important hydrophobic contribution. But, as seen in Fig. 1, an increase in entropy of binding can also arise from dehydration accompanying the formation of electrostatic interactions.

To examine the matter further, we have now calculated the apolar surface areas of the ligands in Table 1, in other words the surface area of the apolar portions in the molecules. Such surface areas (or partial molal volumes, see Cohen and Haberman [17]) are of interest because they are believed to provide a measure of the extent to which the ligands can interact hydrophobically with a binding site. As seen in Fig. 3, the entropy change for the fifteen antagonists is fairly well correlated with the surface area ($r = 0.80$). A distinct relationship is apparent for the four agonists ($r = 0.87$), although the number of observations is far too limited to prove that such a relationship is more than fortuitous.

Physical interpretation of the binding process to D-2 receptors

From Fig. 3 it can be concluded that the larger the apolar surface area of a D-2 receptor ligand, the larger the entropy increase occurring during binding. But the correlation is far from good, neglecting at this stage the fact that two relationships are seen. As a first explanation for such a partial correlation, it is obvious that the apolar surface does not contribute in its totality to hydrophobic bonding, and that the proportion of this surface engaged in hydrophobic

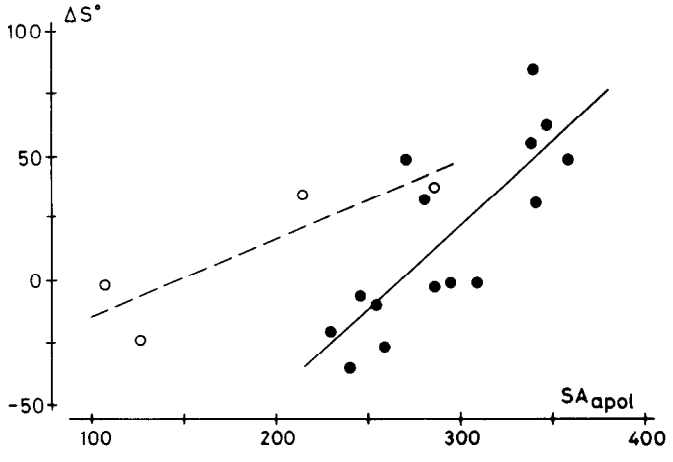

Fig. 3. Correlation between apolar surface area (SA_{apol}) and observed entropy change ($\Delta S°$ at 4°) for the binding to the dopamine D-2 receptor. Key: (○) agonists; and (●) antagonists.

interactions with the receptor certainly varies from one ligand to the other. Butaclamol is particularly noteworthy in this respect since a significant portion of the molecule does not interact with the dopamine receptor [18].

As a second explanation for the findings in Fig. 3, we must remember from Fig. 1 that an increase in entropy can also be contributed by dehydration accompanying the formation of electrostatic bonds, thus justifying a lack of excellent correlation between apolar surface area and increase in entropy. But more important, this mechanism may perhaps afford a preliminary explanation for the distinct behaviour of agonists. Indeed, and with all due reservations for the small number of observations, Fig. 3 would suggest that the contribution of electrostatic bonds to the increase in entropy is larger for agonists than for antagonists. This observation, if confirmed by extensive studies, can have deep implications for discriminating the molecular mechanisms of action of agonists and antagonists.

But what about enthalpy changes? All compounds in Table 1 possess polar groups, in particular a basic amino function. Hence, according to the model shown in Fig. 1, we should expect marked negative enthalpy changes for all these ligands. Table 1 shows that this expectation is not met since the more lipophilic compounds display little or unfavourable enthalpy changes upon binding. Does this mean that for such highly lipophilic ligands electrostatic interactions become small or even unfavourable due to steric reasons (for example a distorsion of steric fit between complementary polar groups)? Such an explanation does not appear reasonable, and it may now be useful to remember the excellent enthalpy–entropy compensation effect noted above for this series of compounds. Such an effect could well indicate a non-molecular or even artefactual origin to unfavourable enthalpy changes, but no conclusion can be drawn until enthalpy–entropy compensation effects in drug–receptor interactions are better understood.

COMPARISON WITH THERMODYNAMIC STUDIES OF OTHER RECEPTORS

In collaboration with another group, some of us have investigated recently the binding of agonists and antagonists to rat lung β-adrenoceptors [19], again finding that entropy and enthalpy contributions are a function of the lipophilicity of the ligands. In this case, however, a parabolic relationship between ΔS° and lipophilicity was found ($r = 0.77$), suggesting in particular different structure–binding relationships between the dopaminergic and β-adrenergic receptors.

In the introduction, we mentioned the work of Hitzemann *et al.* [8] who when studying opiate receptors found entropy-driven binding for the agonist etorphine and enthalpy-driven binding for the antagonist diprenorphine. It can now be seen that these observations are fully in line with our own results since the C-7 alkyl substituent in the etorphine molecule (R = *n*-propyl) is more lipophilic than that in diprenorphine (R = methyl). In other words, etorphine will form a tighter hydrophobic bond with the receptor than diprenorphine, as suggested by Cho *et al.* [20].

CONCLUSION

The above examples and discussion lead to a number of (partly pessimistic) conclusions and (hopefully) constructive proposals regarding both the molecular and pharmacological interpretation of thermodynamic data.

(A) The thermodynamic features of drug–receptor binding appear to be influenced markedly by the lipophilicity of the ligands (as expressed by lipophilic parameters and apolar surface areas or volumes), suggesting a dominant role for hydrophobic bonds. Such a role may be real, it may be an artefact of enthalpy–entropy compensations (see below), or it may be an artefact resulting from the properties of the labeled ligand being displaced. [^{3}H]Spiperone, the ligand used to labeled D-2 receptors in the study discussed above [10], is a highly lipophilic compound with a large apolar surface area (see Table 1). Repeating the study using a more polar ligand (e.g. [^{3}H]sulpiride) will be necessary before further speculating about hydrophobic bonds and their hypothetical dominant role in drug–receptor interactions.

(B) It was concluded earlier in this commentary that the enthalpic and entropic contribution of electrostatic interactions appear to differ between hydrophilic and lipophilic ligands as a result of enthalpy–entropy compensations. Because the significance of such compensation effects in drug–receptor interactions is far from being fully understood, no unambiguous and irrefutable mechanistic interpretation of thermodynamic binding data can yet be given. Theoretical progress appears warranted in this field.

(C) The current belief that the receptor binding of agonists and antagonists is driven by different thermodynamic forces has been disproved for the dopamine D-2 receptor [10] and probably also for the β-adrenoceptor [19]. To assess the situation for other receptors, studies should be undertaken using series of agonists and antagonists with overlapping lipophilicities. However, Fig. 3 brings a first indication that subtle thermodynamic differences between agonist and antagonist binding may nevertheless exist. Such an observation is still too limited to be given credence, but it should be intriguing enough to inspire investigators.

(D) It is generally accepted than an agonist–receptor complex undergoes a conformational change which transfers information and triggers a response, whereas the binding of antagonists blocks conformational changes. Alternatively, it cannot be excluded that different types of conformational changes occur in the receptor–ligand complex, some leading to a response and others being futile. An urgent problem in need of a solution is whether such post-binding events contribute to the enthalpy and entropy changes calculated from receptor affinities (K_i values). The results reported above [10, 19] suggest that the information transfer must occur at a stage energetically distinct from the binding process and independent from its thermodynamic characteristics. This again is a hypothesis in need of confirmation, especially since it runs against current theories.

We believe that this commentary, far from shedding light on the thermodynamic aspects of drug–receptor interactions, indicates that they may be significantly more complex than hitherto assumed. Now is a time for experimenting rather than theorizing. As so aptly put by Jacob Bronowski: "Until a science has passed through a long stage of observation and trial, it cannot develop a system of ordering its observations . . ." [21].

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