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Thermodynamics of antagonist binding to rat muscarinic M_2 receptors: antimuscarinics of the pridinol, sila-pridinol, diphenidol and sila-diphenidol type

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1 We studied the effect of temperature on the binding to rat heart M_2 muscarinic receptors of antagonists related to the carbon/silicon pairs pridinol/sila-pridinol and diphenidol/sila-diphenidol (including three germanium compounds) and six structurally related pairs of enantiomers [(**R**)- and (**S**)-procyclidine, (**R**)- and (**S**)-trihexyphenidyl, (**R**)- and (**S**)-tricyclamol, (**R**)- and (**S**)-trihexyphenidyl methiodide, (**R**)- and (**S**)-hexahydro-diphenidol and (**R**)- and (**S**)-hexbutinol]. Binding affinities were determined in competition experiments using [^{3}H]-N-methyl-scopolamine chloride as radioligand. The reference drugs were scopolamine and N-methyl-scopolamine bromide.

2 The affinity of the antagonists either increased or decreased with temperature. van 't Hoff plots were linear in the 278–310°K temperature range. Binding of all antagonists was entropy driven. Enthalpy changes varied from large negative values (down to -29 kJ mol^{-1}) to large positive values (up to $+30$ kJ mol^{-1}).

3 (**R**)-configurated drugs had a 10 to 100 fold greater affinity for M_2 receptors than the corresponding (**S**)-enantiomers. Enthalpy and entropy changes of the respective enantiomers were different but no consistent pattern was observed.

4 When silanols (R_3SiOH) were compared to carbinols (R_3COH), the affinity increase caused by C/Si exchange varied between 3 and 10 fold for achiral drugs but was negligible in the case of chiral drugs. Silanols induced more favourable enthalpy and less favourable entropy changes than the corresponding carbinols when binding. Organogermanium compounds (R_4Ge) when compared to their silicon counterparts (R_4Si) showed no significant difference in affinity as well as in enthalpy and entropy changes.

5 Exchange of a cyclohexyl by a phenyl moiety was associated with an increase or a decrease in drug affinity (depending on the absolute configuration in the case of chiral drugs) and generally also with a more favourable enthalpy change and a less favourable entropy change of drug binding.

6 Replacement of a pyrrolidino by a piperidino group and increasing the length of the alkylene chain bridging the amino group and the central carbon or silicon atom were associated with either an increase or a decrease of entropy and enthalpy changes of drug binding. However, there was no clear correlation between these structural variations and the thermodynamic effects.

7 Taken together, these results suggest that hydrogen bond-forming OH groups and, to a lesser extent, polarizable phenyl groups contribute significantly to the thermodynamics of interactions between these classes of muscarinic antagonists and M_2 muscarinic receptors.

Keywords: M_2 muscarinic receptors; binding; thermodynamics; van 't Hoff plot; enthalpy; entropy

Introduction

As recently reviewed by Raffa & Porreca (1989) as well as by Hitzemann (1988), thermodynamic analysis of drug-receptor interactions is often used to obtain new information on the differences between agonist and antagonist binding to their receptors. Interest in the temperature-dependence of drug binding was initiated by a study of Weiland *et al.* (1979). These authors observed that agonist binding to turkey erythrocyte β-adrenoceptors is far more temperature-dependent than antagonist binding. They interpreted this observation as indication that the processes leading to information transfer (conformational change of the receptor, receptor-effector interaction, etc.) are driven by enthalpy changes. In contrast with these results, we and others (Barlow *et al.*, 1979; Barlow & Burston, 1979; Waelbroeck *et al.*, 1985; Gies *et al.*, 1986; Muzio *et al.*, 1986) did not observe any coherent pattern when comparing agonist and antagonist binding to various muscarinic receptor subtypes: positive or negative enthalpy changes of binding were observed independently of the drugs' intrinsic efficacy. As explained below, we suspected that the great variation of $\Delta H°'$ and $\Delta S°'$ characterizing antagonist binding to cardiac M_2 muscarinic receptors might reflect differences in the forces driving the drug-receptor interaction (Waelbroeck *et al.*, 1985).

Hoping to identify the contribution of such forces, we investigated in this work the effect of temperature on the binding to rat cardiac muscarinic M_2 receptors of a family of muscarinic antagonists structurally related to the carbon/silicon pairs pridinol/sila-pridinol and diphenidol/sila-diphenidol (see Figure 1), including enantiomerically pure stereoisomers in the case of some chiral drugs. The selective binding and functional affinities of most of the drugs under investigation to M_1–M_4 receptors have been published elsewhere (Eltze *et al.*, 1988; Lambrecht *et al.*, 1988; 1989a,b; Feifel *et al.*, 1990; Dörje *et al.*, 1991; Waelbroeck *et al.*, 1989a,b; 1990a,b; 1991a,b; 1992). The results of functional

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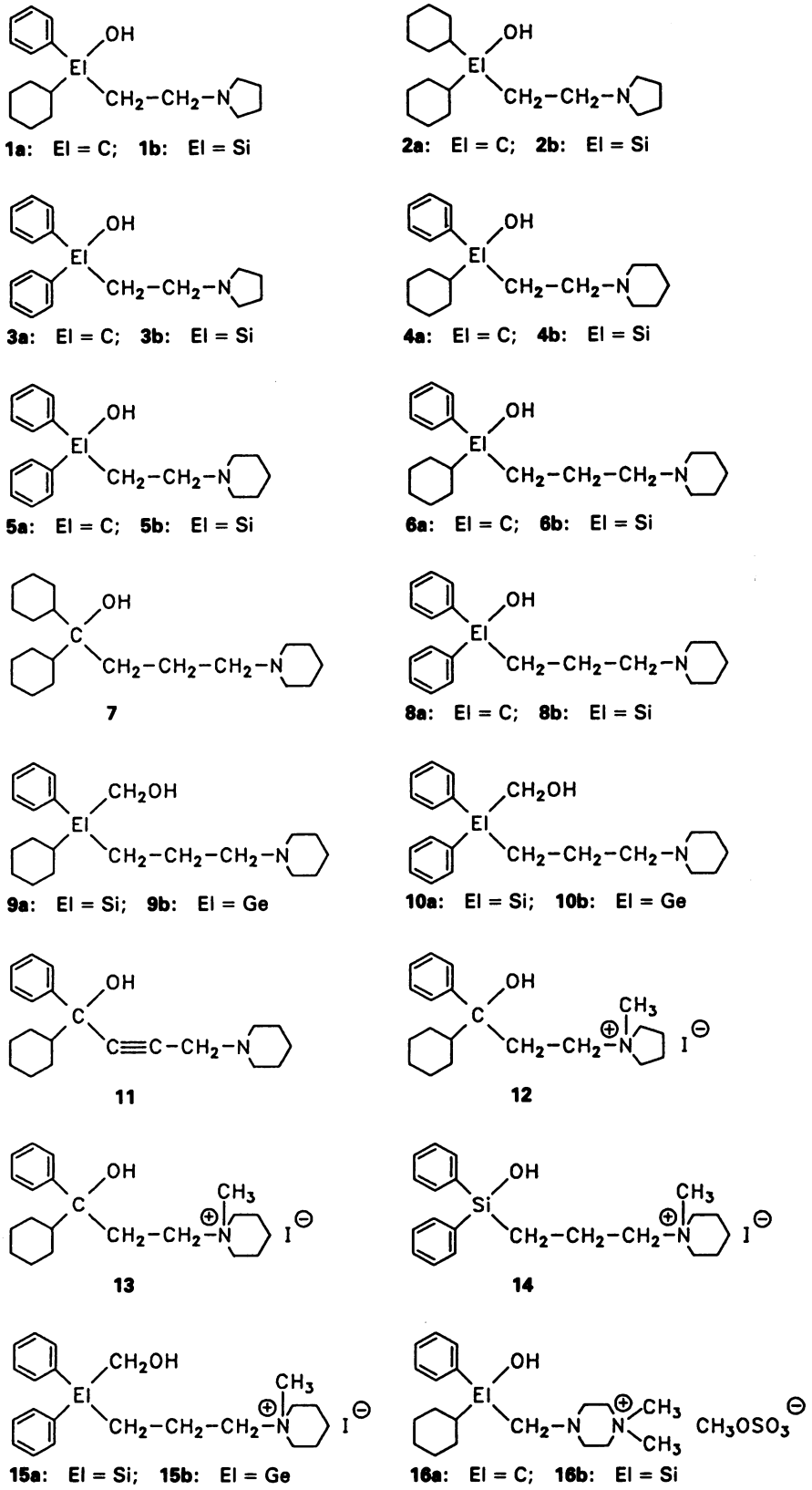

Figure 1 Chemical structure of the antagonists tested in this study (for drug names, see Tables 1 and 2).

studies (Eltze *et al.*, 1988; Lambrecht *et al.*, 1988; 1989a,b, and unpublished work; Waelbroeck *et al.*, 1989b; Feifel *et al.*, 1990) showed that all ligands used were devoid of any agonist activity. The studies described here are related to our systematic investigations on carbon/silicon bioisosterism (for a recent review, see Tacke & Linoh, 1989). Scopolamine and N-methyl-scopolamine bromide were used as reference drugs.

The compounds listed in Figure 1 were chosen to exemplify the chemical and physicochemical properties that might be of importance for enthalpy and entropy effects during drug-receptor interactions. Among the structural parameters were: absolute configuration, C/Si and Si/Ge exchange, cyclohexyl/phenyl exchange, variation in the amino group, variation of the side chain.

We chose to use a rat heart preparation for two reasons: (1) Rat heart contains only M_2 receptors, one of the five subtypes of muscarinic receptors which have been described in mammalian tissues (Hulme *et al.*, 1990; Dörje *et al.*, 1991). (2) The association and dissociation rate constants of antagonists binding to M_2 muscarinic receptors are greater than to other subtypes (M_1, M_3 or M_4) (Waelbroeck *et al.*, 1986; 1991c). It is therefore easier to achieve equilibrium binding in this system, especially at low temperatures. Binding affinities of antagonists were determined in competition experiments by the use of [^{3}H]-N-methyl-scopolamine chloride ([^{3}H]-NMS) as radioligand.

Methods

Cardiac homogenate preparation

Male Wistar Kyoto rats (200–250 g) were killed by decapitation, the heart immediately removed and rinsed in 150 mM NaCl. The homogenization buffer consisted of 20 mM Tris-HCl (pH 7.5) enriched with 250 mM sucrose. Each heart was homogenized in 2 ml of this buffer with an Ultraturrax homogenizer (maximal speed, 5 s at 4°C) followed by the addition of 13 ml of the same buffer, and 7 up and down strokes in a glass-Teflon homogenizer at 4°C. The resulting homogenate was filtered on two layers of medical gauze and either used fresh or stored in liquid nitrogen until use (with no change of muscarinic binding properties).

The protein concentration was determined according to Lowry *et al.* (1951) with bovine serum albumin as standard. The final protein concentration was 400 to 500 μg protein per assay, corresponding to about 250 pM muscarinic binding sites.

Binding assay

[^{3}H]-NMS binding was measured in a total volume of 1.2 ml, in a 50 mM sodium phosphate buffer (pH 7.4) enriched with 2 mM $MgCl_2$. The incubation periods chosen were 4 h at 4°C, 2 h at 12°C, 1 h at 25°C and 20 min at 37°C. [^{3}H]-NMS saturation curves were obtained with different tracer concentrations from 0.25 nM to 4.0 nM. The tracer concentration for competition experiments was 1.0 nM at all temperatures.

To terminate the incubation, each sample was diluted with 2 ml of ice-cold 50 mM sodium phosphate buffer (pH 7.4) and filtered on GF/C glass fibre filters (Whatman, Maidstone, England) presoaked in 0.05% polyethylenimine. The filters were rinsed three times with the same buffer, dried, and the radioactivity counted by liquid scintillation.

Non-specific binding was defined as [^{3}H]-NMS binding in the presence of 1 μM atropine.

Controls

As reported in our previous study using cardiac membranes rather than homogenates (Waelbroeck *et al.*, 1985), the total receptor concentration was not affected by temperature; the half-life of dissociation of the [^{3}H]-NMS-receptor complex varied between approximately 20 min at 5°C and less than 1 min at 37°C. The incubation times chosen in this study were well over 5 dissociation half-lives, and therefore sufficient to allow full equilibration of the binding reaction. The temperature varied by at most ± 1°C during the incubation.

Mathematical analysis and statistics

As found in our previous study (Waelbroeck *et al.*, 1985) the [^{3}H]-NMS Scatchard plots (Scatchard, 1949) were linear at all temperatures studied. The average K_D values of [^{3}H]-NMS were not significantly different from K_D values in our previous study, at the four temperatures used (Waelbroeck *et al.*, 1985).

The Hill coefficients of antagonist competition curves were not significantly different from 1.0, supporting the hypothesis that rat heart muscarinic receptors are homogeneous. Competition curves were therefore analysed assuming the existence of a single receptor class, by use of a computer assisted curve fitting procedure (Richardson & Humrich, 1984). K_i values were calculated from IC_{50} estimates using the Cheng & Prusoff (1973) equation. Affinity constants, as opposed to dissociation constants should be used to evaluate the enthalpy and entropy changes associated with binding; we therefore calculated the K_A values as $1/K_i$, the log K_A value being in fact equal to pK_i. All experiments were repeated 3 times at four temperatures. To estimate the values of the $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$, we plotted the ln K_A values as a function of $1/T$, T being the absolute incubation temperature (van 't Hoff plots). All results were compatible with linear van 't Hoff plots. The enthalpy change ($\Delta H^{\circ\prime}$) due to the binding reaction was derived from the slope of this line, and the entropy change ($\Delta S^{\circ\prime}$) from its ordinate intercept, as shown by the following equation:

$$\ln K_A = -\frac{\Delta H^{\circ\prime}}{R}\frac{1}{T} + \frac{\Delta S^{\circ\prime}}{R}$$

We obtained very low standard deviations for some of the IC_{50} values; however, we felt that this is an underestimate of the true error, when taking into account the different IC_{50} value estimates obtained for a single drug in different batches of results (i.e. comparing four receptor subtypes rather than four temperatures). We therefore used the highest estimate of the standard deviation to evaluate the statistical significance of our results.

The standard deviation of each IC_{50} value was below 16% of the average value in all cases, corresponding to a ln K_A standard deviation of about 0.16, and are not mentioned in the Tables. Due to the relatively small temperature range accessible to experiment (278 to 310° K), this corresponds to large standard deviation values for enthalpy and entropy changes: ± 5.51 kJ mol^{-1} for $\Delta H^{\circ\prime}$ and ± 18.6 J mol^{-1} K^{-1} for $\Delta S^{\circ\prime}$ (assuming no error in tracers K_D values used to calculate K_A values). We used this standard deviation estimate to calculate the standard deviation of the slope and ordinate intercept of the van 't Hoff plot (proportional respectively to $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$), as explained by Colquhoun (1971). Using these estimates, we considered the differences of $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$ as statistically significant if they were, respectively, greater than 8.6 kJ mol^{-1} and 29.8 J mol^{-1} K^{-1} ($P > 0.90$); 11.0 kJ mol^{-1} and 37.3 J mol^{-1} K^{-1} ($P > 0.95$) or 13.2 kJ mol^{-1} and 44.7 J mol^{-1} K^{-1} ($P > 0.98$). This is indicated in the tables as (*$P > 0.90$), (**$P > 0.95$) or (***$P > 0.98$).

Compounds

Compounds were chosen to exemplify chemical and physicochemical properties which might determine the entropic and enthalpic effects of drug-receptor interactions.

Absolute configuration Some of the drugs listed in Figure 1 are chiral, containing a carbon, silicon or germanium atom as the center of chirality. We used the configurationally stable (**R**)- and (**S**)-enantiomers of the carbinols, $R^1R^2R^3COH$, to assess the importance of absolute configuration for the enthalpy and entropy changes of binding. Most of these carbinols were also studied as racemates. Since we have indications that structurally analogous silanols, $R^1R^2R^3SiOH$, may racemize in aqueous solution (Tacke *et al.*, 1987a) these drugs were only tested as racemates. The chiral (hydroxymethyl)silanes, $R^1R^2R^3SiCH_2OH$, and the chiral (hydroxymethyl)germanes, $R^1R^2R^3GeCH_2OH$, were also studied as racemates.

C/Si and Si/Ge exchange Carbon, silicon and germanium are members of Group IV of the Periodic Table. Carbon compounds $R^1R^2R^3R^4C$ may differ from their sila-analogues, $R^1R^2R^3R^4Si$, and germa-analogues, $R^1R^2R^3R^4Ge$, in several respects: (1) Due to the different covalent radii of carbon (0.77Å), silicon (1.17Å) and germanium (1.22Å), the analogues $R^1R^2R^3R^4El$ (El = C, Si, Ge) may differ in their surface (and thus in their lipophilicity) and in their conformational flexibility (C < Si < Ge); (2) Since carbon has a greater electronegativity than silicon and germanium (C > Si ≈ Ge), the carbon compounds may differ somewhat from their sila- and germa-analogues in their bond polarizations; (3) A special effect observed upon C/Si exchange (sila-substitution) of tertiary carbinols $R^1R^2R^3COH$ is an increase in OH acidity. Thus the silanols $R^1R^2R^3SiOH$ are more acidic than the carbinols and may form stronger hydrogen bonds with electron donors (Tacke & Linoh, 1989).

Generally, the chemical and physicochemical properties of silicon and germanium analogues are considerably closer to each other than to their carbon analogues (Lukevics & Ignatovich, 1992).

Cyclohexyl/phenyl exchange The cyclohexyl and phenyl group differ only slightly in size, but the phenyl moiety is considerably more polarizable than the cyclohexyl group.

Variation of the amino group Replacement of the pyrrolidino by a piperidino group as well as N-methylation leads to an increase in size.

Variation of the side chain Elongation of the El-CH_2CH_2-N chain (El = C, Si) by an additional methylene group (→El-$CH_2CH_2CH_2$-N) leads to an increase in size and conformational flexibility.

Scopolamine hydrobromide and N-methyl-scopolamine bromide were obtained from Merck (Darmstadt, Germany). Racemic procyclidine hydrochloride (*1a*.HCl) was provided by Deutsche Wellcome (Burgwedel, Germany). The (**R**)- and (**S**)-enantiomers of procyclidine [(**R**)- and (**S**)-*1a*] (Tacke *et al.*, 1986) as well as racemic sila-procyclidine (*1b*) (Tacke *et al.*, 1983; 1987b) were synthesized according to the literature. Dicyclidine (*2a*) and sila-dicyclidine (*2b*) were obtained by catalytic hydrogenation of pyrrinol (*3a*), and sila-pyrrinol (*3b*), respectively (Tacke *et al.*, unpublished work). Pyrrinol (*3a*) (Adamson, 1949), sila-pyrrinol (*3b*) (Tacke *et al.*, 1984), racemic sila-trihexyphenidyl (*4b*) (Tacke *et al.*, 1983; 1987b), pridinol (*5a*) (Adamson, 1949), sila-pridinol (*5b*) (Tacke *et al.*, 1980; 1984), racemic hexahydro-diphenidol hydrochloride (*6a*.HCl) (Tacke *et al.*, 1985), (**R**)- and (**S**)-hexahydro-diphenidol hydrochloride [(**R**)- and (**S**)-*6a*.HCl] (Tacke *et al.*, 1989a), and racemic hexahydro-sila-diphenidol hydrochloride (*6b*.HCl) (Tacke *et al.*, 1987b) were also synthesized according to published procedures. Racemic trihexyphenidyl hydrochloride (*4a*.HCl) was obtained from Sigma Chemical Co (St Louis, MO, U.S.A.), and (**R**)- and (**S**)-trihexyphenidyl hydrochloride [(**R**)- and (**S**)-*4a*.HCl] were a gift from Dr Aasen (Oslo, Norway). Dicyclidol hydrochloride (*7*.HCl) was obtained by analogy to the synthesis of (**R**)- and (**S**)-hexahydro-diphenidol hydrochloride, starting from dicyclohexyl ketone (Tacke *et al.*, unpublished work). Diphenidol (*8a*) (Miescher & Marxer, 1946) and sila-diphenidol (*8b*) (Steiling *et al.*, 1979; Tacke *et al.*, 1979) were prepared according to the literature. The racemic (hydroxymethyl)silane *9a*, the (hydroxymethyl)silane *10a*, the racemic (hydroxymethyl)germane *9b* and the (hydroxymethyl)germane *10b* were prepared (as hydrochlorides) according to the reaction sequence outlined in the literature (Tacke *et al.*, 1991) for the synthesis of *9a* (starting materials: trichloro(chloromethyl) silane and trichloro(chloromethyl)germane, respectively (Tacke *et al.*, unpublished work). (**R**)- and (**S**)-hexbutinol [(**R**)- and (**S**)-*11*] (Tacke *et al.*, 1989a) and (**R**)- and (**S**)-tricyclamol iodide [(**R**)- and (**S**)-*12*] (Tacke *et al.*, 1986) were synthesized according to the literature. (**R**)- and (**S**)-trihexyphenidyl methiodide [(**R**)- and (**S**)-N-methyl-trihexyphenidyl iodide, (**R**)- and (**S**)-*13*] were gifts from Dr Aasen (Oslo, Norway). Sila-diphenidol methiodide (N-methyl-sila-diphenidol iodide, *14*) (Steiling *et al.*, 1979) was synthesized according to the literature. The methiodides *15a* and *15b* were obtained by quaternization of the corresponding free bases *10a* and *10b*, respectively, with methyl iodide (Tacke *et al.*, unpublished work). Racemic hexocyclium methyl sulphate (*16a*) (Zaugg *et al.*, 1958) and racemic sila-hexocyclium methyl sulphate (*16b*) (Tacke *et al.*, 1989b) were synthesized according to the literature.

[^{3}H]-NMS (1-[N-methyl-^{3}H]-methyl-scopolamine chloride (74 Ci mmol^{-1}) was obtained from Amersham International (Bucks, England). Polyethylenimine was obtained from Sigma Chemicals Co (St Louis, MO, U.S.A.). All other chemicals were of the highest grade available and used as purchased.

Results

General remarks

As previously observed for selective muscarinic antagonists (Waelbroeck *et al.*, 1986; 1990b) the Hill coefficients of competition curves were not significantly different from 1.0 and [^{3}H]-NMS Scatchard plots were linear (not shown). This confirmed that rat heart muscarinic receptors were homogeneous (M_2) receptors. The log K_A values of the pridinol/sila-pridinol and diphenidol/sila-diphenidol type drugs studied

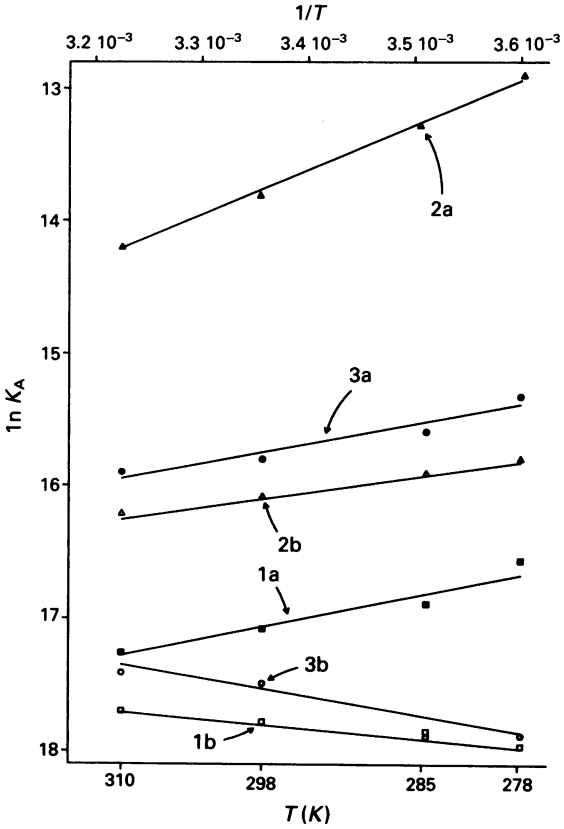

Figure 2 van 't Hoff plots of binding of procyclidine (*1a*), sila-procyclidine (*1b*), dicyclidine (*2a*), sila-dicyclidine (*2b*), pyrrinol (*3a*), and sila-pyrrinol (*3b*). The equilibrium association constants of procyclidine (*1a*) (■), sila-procyclidine (*1b*) (□), dicyclidine (*2a*) (▲), sila-dicyclidine (*2b*) (△), pyrrinol (*3a*) (●), and sila-pyrrinol (*3b*) (○) were calculated as explained in Methods, at four temperatures. ln (K_A) was then plotted as a function of the reciprocal of the absolute temperature. Each point is the average of three determinations in duplicate. The lines were drawn with the $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$ values calculated as indicated in the Methods section.

here varied between 5.8 and 8.9, depending on the drug structure and, in the case of chiral compounds, on the absolute configuration (Tables 1 and 2).

When the effects of temperature on the binding of unlabelled drugs were plotted according to van 't Hoff, linear plots were obtained suggesting that $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$ were constant in the temperature-range studied. This is illustrated in Figure 2 for representative antagonists. $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$ values obtained from these van 't Hoff plots are summarized in Table 1 (racemic and achiral drugs) and 2 (enantiomerically pure chiral drugs). $\Delta H^{\circ\prime}$ values varied between -28.9 kJ mol^{-1} (for scopolamine binding) to $+29.6$ kJ mol^{-1} [for (**R**)-*6a*]. $\Delta S^{\circ\prime}$ values were all positive and varied between $+70$ J mol^{-1} K^{-1} (scopolamine) and $+236$ J mol^{-1} K^{-1} [(**R**)-*6a*]. There was no correlation between the affinity of antagonists for cardiac M_2 muscarinic receptors and the enthalpy or entropy changes due to drug-receptor interaction.

We already know that sila-substitution affects differently the affinity of the (**R**)- and (**S**)-enantiomers of procyclidine [(**R**)- and (**S**)-*1a*] for cardiac muscarinic receptors, and that silanol derivatives may racemize in aqueous solution (for functional studies with the enantiomers of sila-procyclidine [(**R**)- and (**S**)-*1b*] see: Tacke *et al.*, 1987a). The values of $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$ of binding of the enantiomers of chiral silanols are therefore difficult to evaluate with precision. Theoretically, if the (**S**)-enantiomers are unable to recognize muscarinic receptors, the log K_A values of racemic compounds should be underestimated by 0.3 log units [as compared to the active (**R**)-enantiomers] at all temperatures. Since $\Delta H^{\circ\prime}$ measures the temperature dependence of ln K_A (slope of the van 't Hoff plot), the $\Delta H^{\circ\prime}$ of the racemic compounds and of the corresponding active (**R**)-enantiomer should be identical. By contrast, since all log K_A values could be underestimated by 0.3 log units, resulting in an error in the determination of the ordinate intercept of van 't Hoff plots, the $\Delta S^{\circ\prime}$ of racemic drugs could be underestimated by R.ln2 = 5.8 J mol^{-1} K^{-1}. In fact, the log K_A, $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$ of binding of racemic procyclidine (*1a*), trihexyphenidyl (*4a*) and hexahydro-diphenidol (*6a*) were similar to the log K_A, $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$ obtained using their (**R**)-enantiomers (Tables 1 and 2) and we did not observe the expected differences of log K_A and $\Delta S^{\circ\prime}$. This was probably due, in part, to the weak but significant binding affinity of (**S**)-enantiomers to cardiac muscarinic receptors: the stereoselectivity of cardiac receptors for these drugs was 'only' 10 to 100 fold (Table 2), suggesting that the less potent enantiomer could participate in drug binding.

By analogy with these results with carbinols (R_3COH), we considered that the experimental $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$ of binding of analogous racemic silanols (R_3SiOH) were reasonable estimates for the thermodynamic parameters of binding of their active (**R**)-enantiomers.

Absolute configuration

Comparison of the binding properties of the corresponding (**R**)- and (**S**)-enantiomers indicated that the enthalpy and entropy changes associated with drug binding (Table 2) could be markedly affected by the absolute configuration of the drugs.

Effect of sila-substitution

Sila-substitution increased the drug affinity for the receptor (Table 3), with the exception of the carbon/silicon pairs trihexyphenidyl/sila-trihexyphenidyl (*4a/4b*), hexahydro-diphenidol/hexahydro-sila-diphenidol (*6a/6b*) and hexocyclium/sila-hexocyclium (*16a/16b*). In all cases, C/Si exchange led to negative $\Delta\Delta H^{\circ\prime}$ and $\Delta\Delta S^{\circ\prime}$ values, significantly so for five out of eight pairs.

Silicon/germanium exchange

Substitution of the silicon by a germanium atom was associated with small non-significant decreases of the drugs' affinities and of $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$ values (Table 4).

Table 1 Values of log K_A data[a] at 25°C and of the enthalpy and entropy changes associated with the binding of several racemic or achiral antagonists to rat cardiac (M_2) muscarinic receptors, measured by van 't Hoff analysis

Drugs No.	*Name*	*log* K_A	*Enthalpy changes* ($\Delta H^{\circ\prime}$) (kJ mol^{-1})	*Entropy changes* ($\Delta S^{\circ\prime}$) (J mol^{-1} K^{-1})
1a	Procyclidine	7.42	+ 14.6	+ 191
1b	Sila-procyclidine	7.72	− 5.8	+ 128
2a	Dicyclidine	5.99	+ 28.1	+ 209
2b	Sila-dicyclidine	6.98	+ 9.5	+ 166
3a	Pyrrinol	6.85	+ 11.6	+ 170
3b	Sila-pyrrinol	7.61	− 8.8	+ 116
4a	Trihexyphenidyl	7.86	+ 8.9	+ 180
4b	Sila-trihexyphenidyl	7.61	+ 2.8	+ 155
5a	Pridinol	7.50	+ 3.9	+ 156
5b	Sila-pridinol	7.76	− 4.7	+ 130
6a	Hexahydro-diphenidol	6.85	+ 26.8	+ 222
6b	Hexahydro-sila-diphenidol	6.68	+ 10.0	+ 162
7	Dicyclidol	6.06	+ 22.5	+ 193
8a	Diphenidol	6.42	+ 15.2	+ 175
8b	Sila-diphenidol	7.01	− 4.8	+ 118
9a		6.81	+ 9.7	+ 164
9b		6.31	+ 7.5	+ 148
10a		6.16	+ 8.1	+ 146
10b		6.12	+ 4.9	+ 133
14	N-methyl-sila-diphenidol iodide	8.01	− 19.1	+ 90
15a		6.78	− 9.5	+ 98
15b		6.73	− 12.4	+ 87
16a	Hexocyclium methyl sulphate	7.61	+ 3.0	+ 154
16b	Sila-hexocyclium methyl sulphate	7.50	− 4.0	+ 130

[a]The standard deviation of log K_A was about 0.10 and the standard deviation of the estimates of $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$ approximately $\pm$ 5.51 kJ mol^{-1} and $\pm$ 18.6 J mol^{-1} K^{-1}, respectively (see text: Mathematical analysis and statistics).

Table 2 Values of log K_A at 25°C and of the enthalpy and entropy changes associated with the binding of the (R)- and (S)-enantiomers of several antagonists to rat cardiac (M_2) muscarinic receptors, measured by van 't Hoff analysis[a]

Drugs No.	*Name*	log K_A	*Enthalpy changes* ($\Delta H^{\circ\prime}$) (kJ mol^{-1})	*Entropy changes* ($\Delta S^{\circ\prime}$) (J mol^{-1} K^{-1})
(R)-*1a*	(R)-Procyclidine	7.58	+ 13.6	+ 190
(S)-*1a*	(S)-Procyclidine	5.76	+ 18.5	+ 172
(R)-*4a*	(R)-Trihexyphenidyl	7.81	+ 8.5	+ 178
(S)-*4a*	(S)-Trihexyphenidyl	6.01	+ 13.8	+ 162
(R)-*6a*	(R)-Hexahydro-diphenidol	7.08	+ 29.6	+ 236
(S)-*6a*	(S)-Hexahydro-diphenidol	5.81	+ 21.4	+ 183***
(R)-*11*	(R)-Hexbutinol	7.98	− 5.4	+ 134
(S)-*11*	(S)-Hexbutinol	7.15	+ 23.8***	+ 217***
(R)-*12*	(R)-Tricyclamol iodide	8.38	+ 18.2	+ 221
(S)-*12*	(S)-Tricyclamol iodide	6.61	+ 12.1	+ 167***
(R)-*13*	(R)-N-methyl-trihexyphenidyl iodide	8.92	+ 14.3	+ 218
(S)-*13*	(S)-N-methyl-trihexyphenidyl iodide	6.98	+ 7.7	+ 159***
	Scopolamine	8.76	− 28.9	+ 70
	N-methyl-scopolamine bromide	9.53	− 26.9	+ 92

[a]The standard deviation of log K_A was about 0.10 and the standard deviation of the estimates of $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$ approximately ± 5.51 kJ mol^{-1} and ± 18.6 J mol^{-1} K^{-1}, respectively (see text: Mathematical analysis and statistics).
***Significantly different ($P > 0.98$) from entropy (enthalpy) change associated with binding of the (R)-enantiomer.

Table 3 Differences between the log K_A, $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$ values observed when comparing the binding of structurally analogous carbinols and silanols (C/Si exchange, sila-substitution)

Drugs	Δ log K_A	$\Delta\Delta H^{\circ\prime}$ (kJ mol^{-1})	$\Delta\Delta S^{\circ\prime}$ (J mol^{-1} K^{-1})
Procyclidine (*1a*)/ Sila-procyclidine (*1b*)	+ 0.30	− 20.4***	− 63***
Dicyclidine (*2a*)/ Sila-dicyclidine (*2b*)	+ 0.99	− 18.6***	− 43**
Pyrrinol (*3a*)/Sila-pyrrinol (*3b*)	+ 0.76	− 20.4***	− 54***
Trihexyphenidyl (*4a*)/ Sila-trihexyphenidyl (*4b*)	− 0.25	− 6.1	− 25
Pridinol (*5a*)/Sila-pridinol (*5b*)	+ 0.26	− 8.6*	− 26
Hexahydro-diphenidol (*6a*)/ Hexahydro-sila-diphenidol (*6b*)	− 0.17	− 16.8***	− 60***
Diphenidol (*8a*)/Sila-diphenidol (*8b*)	+ 0.59	− 20.0*	− 57***
Hexocyclium methyl sulphate (*16a*)/ Sila-hexocyclium methyl sulphate (*16b*)	− 0.11	− 7.0	− 24

Statistically significant: *$P > 0.90$; **$P > 0.95$; ***$P > 0.98$.

Table 4 Differences in log K_A, $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$ values observed when comparing the binding of structurally analogous silanes and germanes (Si/Ge exchange)

Drugs	Δ log K_A	$\Delta\Delta H^{\circ\prime}$ (kJ mol^{-1})	$\Delta\Delta S^{\circ\prime}$ (J mol^{-1} K^{-1})
9a/9b	− 0.50	− 2.2	− 16
10a/10b	− 0.04	− 3.2	− 13
15a/15b	− 0.05	− 2.9	− 11

Cyclohexyl/phenyl exchange

Substitution of a cyclohexyl by a phenyl group increased or decreased the drug affinities, depending on the absolute configuration of the chiral compounds (Table 5). Except for the pair dicyclidol/(R)-hexahydro-diphenidol [*7*/(R)-*6a*], negative $\Delta\Delta H^{\circ\prime}$ values were observed when replacing the cyclohexyl by a phenyl moiety, significantly so in eight out of nineteen pairs of molecules. This was compensated by less favourable entropic effects (negative $\Delta\Delta S^{\circ\prime}$ values), statistically significant in seven out of nineteen pairs of drugs.

N-methylation of the cyclic amino group

The effect of N-methylation on log K_A, $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$ values of (R)- and (S)-procyclidine [(R)- and (S)-*1a*], (R)- and (S)-trihexyphenidyl [(R)- and (S)-*4a*], sila-diphenidol [*8b*], *10a* and *10b*, is summarized in Table 6. The log K_A values were increased by up to one log unit, depending on the drugs' structure. The modifications of $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$ were positive with the (R)-enantiomers, negative with the (S)-enantiomers and achiral compounds.

Pyrrolidino/piperidino exchange

Only small and non-significant modifications (of either sign) of the compounds' affinities, and of the $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$ values of binding were observed (Table 7).

Table 5 Differences in log K_A, $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$ values observed when comparing the binding of compounds with a cyclohexyl group and their corresponding phenyl analogues (cyclohexyl/phenyl exchange)

Drugs	Δ *log* K_A	$\Delta\Delta H^{\circ\prime}$	$\Delta\Delta S^{\circ\prime}$
1 Cyclohexyl/phenyl group		(kJ mol^{-1})	(J mol^{-1} K^{-1})
(R)-Procyclidine [(R)-*1a*]/Pyrrinol (*3a*)	− 0.73	− 2.0	− 20
(S)-Procyclidine [(S)-*1a*]/Pyrrinol (*3a*)	+ 1.09	− 6.9	− 2
Sila-procyclidine (*1b*)/ Sila-pyrrinol (*3b*)	− 0.11[a]	− 3.0	− 12
Dicyclidine (*2a*)/ (R)-Procyclidine [(R)-*1a*]	+ 1.59	− 14.5***	− 19
Dicyclidine (*2a*)/ (S)-Procyclidine [(S)-*1a*]	− 0.23	− 9.6*	− 37*
Sila-dicyclidine (*2b*)/ Sila-procyclidine (*1b*)	+ 0.74	− 15.3***	− 38*
(R)-Trihexyphenidyl [(R)-*4a*]/Pridinol (*5a*)	− 0.31	− 4.6	− 22
(S)-Trihexyphenidyl [(S)-*4a*]/Pridinol (*5a*)	+ 1.49	− 9.9*	− 6
Sila-trihexyphenidyl (*4b*)/ Sila-pridinol (*5b*)	+ 0.15[a]	− 7.5	− 25
(R)-Hexahydro-diphenidol [(R)-*6a*]/ Diphenidol (*8a*)	− 0.66	− 14.4*	− 61***
(S)-Hexahydro-diphenidol [(S)-*6a*]/ Diphenidol (*8a*)	+ 0.61	− 6.2	− 8
Hexahydro-sila-diphenidol (*6b*)/ Sila-diphenidol (*8b*)	+ 0.33[a]	− 14.8***	− 44**
Dicyclidol (*7*)/ (R)-Hexahydro-diphenidol [(R)-*6a*]	+ 1.02	+ 7.1	+ 43**
Dicyclidol (*7*)/ (S)-Hexahydro-diphenidol [(S)-*6a*]	− 0.25	− 1.1	− 10
9a/10a	− 0.65[a]	− 1.6	− 18
9b/10b	− 0.19[a]	− 2.6	− 15
2 Cyclohexyl/phenyl groups			
Dicyclidine (*2a*)/Pyrrinol (*3a*)	+ 0.86	− 16.5**	− 39*
Sila-dicyclidine (*2b*)/ Sila-pyrrinol (*3b*)	+ 0.63	− 18.3**	− 50***
Dicyclidol (*7*)/Diphenidol (*8a*)	+ 0.36	− 7.3	− 18

[a]One drug in each pair is a racemic mixture, the second an achiral molecule. The differences in log K_A, $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$ shown in this Table are, therefore, approximate values only (see Results: general remarks).
Statistically significant: *$P>0.90$; **$P>0.95$; ***$P>0.98$.

Table 6 Differences in log K_A, $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$ values observed when comparing the binding of tertiary and the corresponding quaternary (N-methylated) drugs (N-methylation)

Drugs	Δ *log* K_A	$\Delta\Delta H^{\circ\prime}$	$\Delta\Delta S^{\circ\prime}$
		(kJ mol^{-1})	(J mol^{-1} K^{-1})
(R)-Procyclidine [(R)-*1a*]/ (R)-Tricyclamol iodide [(R)-*12*]	+ 0.80	+ 4.6	+ 31*
(S)-Procyclidine [(S)-*1a*]/ (S)-Tricyclamol iodide [(S)-*12*]	+ 0.85	− 6.4	− 5
(R)-Trihexyphenidyl [(R)-*4a*]/ (R)-N-methyl-trihexyphenidyl iodide [(R)-*13*]	+ 1.11	+ 5.8	+ 40**
(S)-Trihexyphenidyl [(S)-*4a*]/ (S)-N-methyl-trihexyphenidyl iodide [(S)-*13*]	+ 0.97	− 6.1	− 3
Sila-diphenidol (*8b*)/ N-methyl-sila-diphenidol iodide (*14*)	+ 1.00	− 14.3***	− 28
10a/15a	+ 0.62	− 17.6***	− 48***
10b/15b	+ 0.61	− 17.3***	− 46***

Statistically significant: *$P>0.90$; **$P>0.95$; ***$P>0.98$.

Side-chain elongation

Elongation of the El-CH_2CH_2-N side chain (El = C,Si) by an additional methylene group led to a decrease by up to one log unit of log K_A values (Table 8). Except for the pair (R)-trihexyphenidyl/(R)-hexahydro-diphenidol [(R)-*4a*/(R)-*6a*] (statistically significant increases of the $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$ values were found), only small and non-significant changes of either sign of $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$ values were observed.

Table 7 Differences in log K_A, $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$ values observed when comparing the binding of pyrrolidino-substituted compounds and their corresponding piperidino analogues (pyrrolidino/piperidino exchange)

Drugs	Δ *log* K_A	$\Delta\Delta H^{\circ\prime}$ (kJ mol^{-1})	$\Delta\Delta S^{\circ\prime}$ (J mol^{-1} K^{-1})
(R)-Procyclidine [(R)-*1a*]/ (R)-Trihexyphenidyl [(R)-*4a*]	+ 0.23	− 5.1	− 12
(S)-Procyclidine [(S)-*1a*]/ (S)-Trihexyphenidyl [(S)-*4a*]	+ 0.25	− 4.7	− 10
Sila-procyclidine (*1b*)/ Sila-trihexyphenidyl (*4b*)	− 0.11	+ 8.6	+ 27
Pyrrinol (*3a*)/Pridinol (*5a*)	+ 0.65	− 7.7	− 14
Sila-pyrrinol (*3b*)/ Sila-pridinol (*5b*)	+ 0.15	+ 4.1	+ 14
(R)-Tricyclamol iodide [(R)-*12*]/ (R)-N-methyl-trihexyphenidyl iodide [(R)-*13*]	+ 0.54	− 3.9	− 3
(S)-Tricyclamol iodide [(S)-*12*]/ (S)-N-methyl-trihexyphenidyl iodide [(S)-*13*]	+ 0.37	− 4.4	− 8

Table 8 Differences in log K_A, $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$ values observed when comparing the binding of compounds with an El-CH_2CH_2-N chain (El = C, Si) and their corresponding analogues containing an El-$CH_2CH_2CH_2$-N moiety (elongation of the side chain)

Drugs	Δ *log* K_A	$\Delta\Delta H^{\circ\prime}$ (kJ mol^{-1})	$\Delta\Delta S^{\circ\prime}$ (J mol^{-1} K^{-1})
(R)-Trihexyphenidyl [(R)-*4a*]/ (R)-Hexahydro-diphenidol [(R)-*6a*]	− 0.73	+ 21.1***	+ 58***
(S)-Trihexyphenidyl [(S)-*4a*]/ (S)-Hexahydro-diphenidol [(S)-*6a*]	− 0.20	+ 7.6	+ 21
Sila-trihexyphenidyl (*4b*)/ Hexahydro-sila-diphenidol (*6b*)	− 0.93	+ 7.2	+ 7
Pridinol (*5a*)/ Diphenidol (*8a*)	− 1.08	+ 11.3	+ 19
Sila-pridinol (*5b*)/ Sila-diphenidol (*8b*)	− 0.75	− 0.1	− 12

Statistically significant: ***$P > 0.98$.

Discussion

The pioneering work of Weiland *et al.* (1979) on β-adrenoceptors led to the hope that agonists and antagonists would generally induce different enthalpy and entropy changes when associating with their receptors (independently of the drug structure or of the receptor studied), so that these differences could reflect the initiation or blockade of information transfer. Subsequent studies however indicated that this generalization was not tenable (Raffa & Porreca, 1989). Several authors therefore suggested that thermodynamic parameters of binding might reflect detailed molecular drug-receptor interactions (Kilpatrick *et al.*, 1986; Hitzemann, 1988; Miklavc *et al.*, 1990).

In an attempt to understand the enthalpic and entropic effects of non-covalent binding, the thermodynamic behaviour of simple model systems has been investigated in great detail (Edelhoch & Osborne, 1976). The forces driving the non-covalent association of two molecules are limited in number (Barrow, 1973; Ross & Subramanian, 1981).

(1) *Ionic bonds* are formed when two groups with opposite charges are brought into close contact. Since free ions interact strongly with water (ion-dipole interactions), the entropy changes associated with ionic bond formation in aqueous solutions are positive (Edelhoch & Osborne, 1976; Ross & Subramanian, 1981). The enthalpy changes are small (Ross & Subramanian, 1981) or positive (Edelhoch & Osborne, 1976). The molecular rearrangement of water being strongly temperature-dependent, the heat capacity change ($\Delta C_p^{\circ\prime}$, variation of $\Delta H^{\circ\prime}$ with temperature) associated with ionic bond formation is positive at 25°C (Edelhoch & Osborne, 1976).

(2) *van der Waals* interactions cover all dipole-dipole attractions. They can be divided into three contributions: (2a) a dipole-dipole attraction term (attraction between two molecules with a permanent dipole); (2b) an induction effect (caused by polarization of the electrons of a molecule when brought into contact with a permanent dipole); and (2c) London dispersion forces, which describe the induction effect of each 'instantaneous' dipole on the adjacent polarizable atom or molecule, allowing induced dipole-induced dipole interactions regardless of whether the interacting atoms or molecules have a permanent dipole or not (Barrow, 1973). In aqueous solution, these interactions are associated with negative enthalpy and entropy changes (Edelhoch & Osborne, 1976).

(3) *Hydrogen binding* occurs when a somewhat acidic hydrogen atom (carrying a partial positive charge) comes into contact with a lone electron pair of an atom, such as an oxygen or nitrogen atom, in a neighbouring molecule (Barrow, 1973). In aqueous solution, these interactions are also associated with negative enthalpy and entropy changes (Edelhoch & Osborne, 1976). Hydrogen bond formation, like hydrophobic interactions, is associated with a negative $\Delta Cp^{\circ\prime}$.

(4) *Hydrophobic interactions* appear when non-polar compounds are brought into contact from water. These binding interactions result from the molecular rearrangement of water, and are therefore strongly temperature-dependent. They are associated with positive enthalpy and entropy changes and (by contrast with ionic bond formation) negative heat capacity changes (Edelhoch & Osborne, 1976).

Our goal in this work was to test the hypothesis that differences in enthalpy and entropy changes, associated with antagonist binding to cardiac muscarinic M_2 receptors, could be explained by differences in hydrogen bonding or van der Waals interactions between the drug and its receptor. We therefore deliberately chose drugs with very similar chemical structures. Essential contributions to the solution of this problem was expected from the study of C/Si and Si/Ge pairs.

Binding of the muscarinic antagonists studied was driven by a large entropy increase, while the enthalpy change associated with binding varied between large positive values and large negative values (this work, Tables 1 and 2; see also Barlow *et al.*, 1979; Barlow & Burston, 1979; Waelbroeck *et al.*, 1985; Gies *et al.*, 1986; Muzio *et al.*, 1986). Few of the differences of $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$ of binding ($\Delta\Delta H^{\circ\prime}$ and $\Delta\Delta S^{\circ\prime}$) observed in this work could be considered as statistically significant, due to the small temperature range accessible to experiment (5 to 37°C), and to the small structural variations studied. Nevertheless, we believe that the results of our thermodynamic analysis provides an insight into the molecular events underlying the interaction of antagonists with muscarinic receptors not obtainable by other experimental techniques. The following conclusions may be drawn:

(1) We did not observe any variation of values of $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$ of binding with temperature. It is known that the cationic head of quaternary ammonium or protonated amino groups of muscarinic antagonists interacts with a negatively charged aspartate residue (Hulme *et al.*, 1990). This residue turns towards a relatively hydrophobic region of the receptor, formed by a bundle of seven transmembrane helices (Hulme *et al.*, 1990). Our results therefore suggest that the heat capacity changes due to hydrophobic, hydrogen, and ionic bond formation approximately compensated each other when the muscarinic antagonist binds to the receptor.

(2) The entropy and enthalpy changes associated with binding of the corresponding (**R**)- and (**S**)-enantiomers were often significantly different (Table 2). This is a reminder that these drugs interact with an asymmetric binding site: thermodynamic parameters reflect the drug-receptor interaction, not the drugs' chemical and physicochemical properties. The differences of affinity of the two enantiomers were below 100 fold for the compounds studied here. This corresponds either to a $\Delta\Delta H^{\circ\prime}$ value of 10.6 kJ mol^{-1} (assuming that the binding $\Delta S^{\circ\prime}$ of the two enantiomers are equal) or to a $\Delta\Delta S^{\circ\prime}$ value of 38 J mol^{-1} K^{-1} (assuming that the binding $\Delta H^{\circ\prime}$ of the two enantiomers are equal). These values would be barely significant. For compounds (**S**)-*1a* and (**S**)-*4a*, the binding $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$ were both less favourable than those of (**R**)-*1a* or (**R**)-*4a* respectively: the $\Delta\Delta H^{\circ\prime}$ and $\Delta\Delta S^{\circ\prime}$ values were not statistically significant. In contrast, for the pairs of enantiomers of *6a*, *11*, *12* and *13*, the weaker (**S**)-enantiomer had either a more favourable $\Delta H^{\circ\prime}$ or a more favourable $\Delta S^{\circ\prime}$ as compared to the more potent (**R**)-enantiomer. This was overcompensated by a very large unfavourable change of $\Delta S^{\circ\prime}$ or $\Delta H^{\circ\prime}$, respectively.

(3) The enthalpy changes associated with binding of silanols (R_3SiOH) were more favourable than those associated with binding of the corresponding carbinols (R_3COH) (Table 3). On the other hand, sila-substitution resulted in less favourable entropic effects. This is in keeping with model systems (Edelhoch & Osborne, 1976; Ross & Subramanian, 1981): silanols are more acidic than carbinols and therefore susceptible to form stronger hydrogen bonds either with an electron donor atom of the receptor or with water, and hydrogen bond formation is associated with negative enthalpy and entropy changes.

(4) Since (hydroxymethyl)silanes (R_3SiCH_2OH) and (hydroxymethyl)germanes (R_3GeCH_2OH) do not differ significantly in OH acidity, lipophilicity and conformational flexibility, Si/Ge exchange did not lead to the thermodynamic effects observed upon C/Si exchange in the carbinol/silanol (R_3COH/R_3SiOH) structures (compare Tables 4 and 3). Generally, in accordance with the covalent radii ($C << Si < Ge$) and electronegativities ($C < Si \approx Ge$) of carbon, silicon and germanium, analogous silicon and germanium compounds are considerably closer by their chemical and physicochemical properties than to their corresponding carbon analogues (Lukevics & Ignatovich, 1992). These similarities in their chemical and physicochemical properties are reflected by the distinct bioisosteric relationships between analogous silicon and germanium compounds (see for example: Tacke *et al.*, 1992).

(5) The enthalpy change associated with binding of phenyl-substituted drugs was systematically more favourable than that of the corresponding cyclohexyl derivatives (Table 5). This was in keeping with results expected from model systems (Edelhoch & Osborne, 1976; Ross & Subramanian, 1981): a phenyl ring is more polarizable than a cyclohexyl group so that the contribution to van der Waals interactions via induction effects and London dispersion forces should be strengthened by substituting a cyclohexyl by a phenyl group.

(6) N-methylation of the tertiary amino group, as well as increasing the size of the heterocycle from pyrrolidino to piperidino, or lengthening the alkylene chain (i.e. changes increasing the hydrophobicity and conformational flexibility of the drugs) did not change the thermodynamic parameters of drug binding in a clear way (compare Tables 6, 7, 8). This is in keeping with model systems (Edelhoch & Osborne, 1976): dissolving hydrophobic molecules in water or their transfer from organic solvents into water (these reactions being equivalent to drug dissociation from the receptor) is associated with negative $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$values, but there is no correlation between the absolute value of $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$ and the size of the hydrophobic molecule (Edelhoch & Osborne, 1976).

Although not extensive, the list of studies which have applied thermodynamic analysis to pharmacological data is growing (Raffa & Porreca, 1989). The data obtained for various receptors demonstrate that there is a wide diversity in the thermodynamic profiles for agonist and antagonist ligands. For some receptors, such as the β-adrenoceptors, several reports are available (Raffa & Porreca, 1989).

As stated in the Introduction, binding of β-adrenoceptor agonists is driven by a large enthalpy decrease, while antagonist binding is associated with an entropy increase (Weiland *et al.*, 1979). This was interpreted as reflecting the receptor activation and interaction with a GTP-binding protein when an agonist is present.

More recently, Strader *et al.* (1989) identified two serine residues which are involved in agonist activation of the β-adrenoceptor. Agonist interaction with the receptor probably involves the formation of specific hydrogen bonds with these serine residues, causing the conformational changes in the receptor which lead to the activation of the GTP-binding protein. Since hydrogen bond formation is associated with the negative enthalpy and entropy changes, hydrogen bonds formation between the receptor and agonists but not between the receptor and antagonists might explain the observation that agonists but not antagonists binding to β-adrenoceptors is enthalpy driven.

Thus, thermodynamic analysis of pharmacological data potentially offers an insight into the molecular events underlying drug-receptor interactions not obtainable by other techniques. It offers the promise of measuring parameters that are more fundamental than equilibrium affinity constants. Implicit in thermodynamic analysis is quantitative measurement of the driving forces involved in the drug-receptor interaction and responsible for the affinity of an antagonist for a receptor and the efficacy of an agonist.

In conclusion, the thermodynamic analysis of the interactions of several muscarinic antagonists of the pridinol, sila-pridinol, diphenidol and sila-diphenidol type with M_2 cardiac receptors suggests that hydrogen bonds and polarizable groups play an important role in determining the enthalpy and entropy changes associated with ligand binding.

The sila-substitution approach (C/Si exchange) proved to be a valuable tool in these studies.

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