Computer-Aided Mapping of the β-Adrenoceptor. 1. Explanation for Effect of Para Substitution on Blocking Activity at the β-1-Adrenoceptor

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Received January 9, 1992

Anomalously low affinities for the β-1-adrenoceptor are seen for members of a series of para-substituted *N*-isopropylphenoxypropanolamines in which the substituent is able to conjugate with the aromatic ring. The energy of conjugation was calculated using the AM1 semiempirical molecular orbital method and appears to correlate with the loss of binding energy, and hence affinity for the receptor. This suggests that binding is associated with movement of the substituent out of the plane of the aromatic ring due to steric interference with the receptor. A previously unrecognized binding site for aromatic groups off the para position is also identified.

Introduction

β-1-Adrenoceptors are membrane-bound glycoproteins coupled to the enzyme adenylate cyclase via a guanine nucleotide regulatory protein. Drugs which block the β-adrenoceptor have been shown to be useful therapeutic agents in a wide range of cardiovascular conditions including ischaemic heart disease and hypertension, whereas agonists are important drugs for the treatment of asthma and other disease states.[1] Lands et al.[2] have subdivided this receptor into β-1 and β-2 subtypes and more recently, the sequencing of the human β-1- and β-2-adrenoceptors has proved this subdivision beyond doubt.[3,4] The β-1/β-2 profile of many agonists and antagonists has now been established and a β-3 receptor has been proposed to explain the thermogenic properties of a new class of β-adrenoceptor agonists.[5]

We have investigated the structure of the β-1-adrenoceptor in its low affinity (antagonist) state. Detailed knowledge of the three-dimensional structure of a receptor offers a direct route for the medicinal chemist intent on designing drugs with optimum qualities. This is of particular relevance to the present generation of β-1-blockers, which are hampered by side effects or low affinity or both.[1] Since such detailed knowledge is not yet available for the β-adrenoceptors, indirect methods must be used to obtain this information. One way is through conformational analyses of compounds active at the desired receptor. Although there is a wide range of compounds which block the β-1-adrenoceptor, the conformational flexibility of these compounds limits the extent to which solid-state,[6-11] solution,[12-15] or theoretical[6,10,11,13,16,17] structures can be related to the biologically active conformation(s). Low activity of conformationally restricted analogues[18-22] has also left doubt as to how relevant derived conformations are to the receptor.

Linschoten et al.[23] have constructed a model of the active site and estimated approximate energies of specific binding points of the β-1-adrenoceptor in the low-affinity state. They used a computational method derived from the distance-geometry approach pioneered by Crippen,[24] in which the problems associated with high flexibility in active

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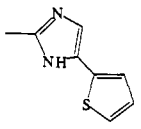
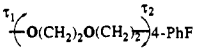
Table I. Structures, Activities, and Some Other Physical Data for the Para-Substituted Aryloxy-3-(isopropylamino)propan-2-ols, 4-$RPhOCH_2CH(OH)CH_2NHCH(CH_3)_2$, Used in This Study

no.	R	name or ref	I pA_2[a]	II pA_2(corr)[b]	III $\Delta G°_{obs}$[c]	IV energy[d] loss	V $\Delta H_f(0°)$[e]	VI $\Delta H_f(90°)$[d]	VII $\Delta H_f(90°) - \Delta H_f(0°)$[g]	VIII hydrophobicity[h]
1	$NHCOCH_3$	practolol	6.0	5.3	−31	10	−220	−206	14	−0.6
2	$CO_2CH_2CH_3$	35	6.5	5.2	−31	10	−442	−429	13	0.8
3	CO_2CHCH_2	35	6.3	5.0	−30	11	−297	−285	12	0.5
4	$O(CH_2)_2OCH_3$	H87/07	6.4	5.7	−34	7	−414	−410	4	0.3
5		33, 34	7.6	5.9	−35	6	160	165	5	0.2
6		33, 34	8.9	7.2	−43	−2	not calculated[i]			no data[j]
7		Ro-31-1118	8.2	7.5	−45	−4	−515	−512	3	2.7
8	$(CH_2)_2OCH_2C_3H_5$	betaxolol	7.4	6.7	−40	1	−192	−195	−3	1.3
9	$(CH_2)_2CO_2CH_3$	35	7.0	5.7	−34	7	−475	−478	−3	0.5
10	CH_2CONH_2	atenolol	6.8	6.1	−36	5	−254	−262	−8	−1.5
11	CH_2CH_3	25	7.2	6.0	−36	5	−120	−122	−2	1.2

[a] Activity at the β-1-receptor. Activities of named molecules were obtained as described in Experimental Section. Others were obtained as described in the original reference. The number of experiments was ≥2 for each compound, and tabulated pA_2 values are mean values. The range for each value is ≤±0.2. [b] Standardized and corrected as explained in Experimental Section. [c] Calculated in kJ mol^{-1} from eqs 1 and 4. [d] Difference between $\Delta G°_{obs}$ and $\Delta G°_{calcd}$, the latter value of which, calculated[23] for the *N*-isopropylphenoxypropanolamine chain, is −41 ± 6 kJ mol^{-1}. [e] Calculated for planar molecules (i.e. side chain R is coplanar with the phenyl ring) in kJ mol^{-1} using AM1,[30] with the propanolamine side chain replaced by a methyl group. [f] Calculated for nonplanar molecules (i.e. side chain R is at 90° to the phenyl ring) in kJ mol^{-1} using AM1,[30] with the propanolamine side chain replaced by a methyl group. [g] For compounds 1–7 this represents the energy of conjugation in kJ mol^{-1}. [h] Estimated for the para substituent using Rekker and de Kort's system.[38] [i] Parameters for sulfur not available in AM1[30] (MOPAC version 5.0). [j] No data on the hydrophobicity of the thiophene fragment using the Rekker and de Kort system.[38]

compounds are minimized through exploitation of a large number of molecules. Explanations of the effects of all but para substitution, for which there was only one representative molecule (practolol, 1 in Table I) in the database, were given. It was of note, however, that even though practolol had significant specificity for the β-1-adrenergic receptor, its activity was anomalously low.

In the interest of developing better β-1-specific drugs, we have concentrated on para-substituted β-blockers. Para-substitution is often accompanied by β-1-specificity as a result of a relative decrease in β-2-receptor affinity, and it has been suggested[25,26] that steric effects in the β-2-receptor, rather than lipophilic effects, are responsible for this. There has been no previous attempt to relate variations in biological activity of different para-substituted β-blockers to the physicochemical nature of the β-1-adrenoceptor. To do so is the aim of this paper.

Results and Discussion

We measured the biological activity of a number of para-substituted β-blockers (molecules 1, 4, 7, 8, and 10, Table I) and extracted the structures and biological activities of a number of others (molecules 2, 3, 5, 6, 9, and 11, Table I) from the literature in which the electronic, lipophilic, and steric natures of the para substituents differed widely (Table I), but which all contained the *N*-isopropylphenoxypropanolamine structure. The method used to standardize the pA_2 values and in turn calculate the binding energies of these compounds is described in detail in the experimental methods.

All molecules were assumed to bind to the β-1-adrenoceptor in the same mode. The common interaction points of the molecules with the receptor being the β-hydroxyl, the amino nitrogen, and the phenyl ring.

The hydrogen-bond-donor/-acceptor properties of the molecules chosen for this study vary widely. Previous studies on practolol indicate that the amide is involved in a hydrogen-bond interaction with the receptor.[27,28] However, this hydrogen-bond interaction does not appear to be essential for cardioselectivity.[25,29] This is illustrated in molecule 11 in Table I. The ethyl para substituent of

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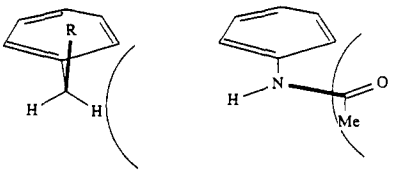

Figure 1. Schematic illustration of how a receptor wall could interfere with substituents which are conjugated with the phenyl ring (right) and not interfere with unconjugated substituents (left).

molecule 11 is unable to donate or accept a hydrogen bond yet it is more active at the β-1-adrenoceptor than practolol (the same argument holds for molecules 8 and 9, which both contain methylene groups in the position corresponding to the amide in practolol).

From Linschoten et al.'s model of the β-1-adrenoceptor active site,[23] the binding energy of the *N*-isopropylphenoxypropanolamine substructure can be calculated. Since all of the compounds 1–11 contain this substructure, the effect of the para-substituent upon the binding energy can be examined. We observed that a common feature of molecules 1–7 was a group which could be conjugated with the phenyl ring. We then hypothesized that steric interaction with the β-1-adrenoceptor wall forces the para substituent out-of-plane with the aromatic ring, and that the energy required could account for the poor binding of these compounds (Figure 1).

The binding energy or $\Delta G°_{calcd}$ for the *N*-isopropylphenoxypropanolamine substructure is −41 kJ mol^{-1} with a 95% confidence interval of 6 kJ mol^{-1}. It can be seen in column III of Table I that for five of the molecules 1–7 this value falls short by 6–11 kJ mol^{-1}, as witnessed by the positive values under column IV, "energy loss". However, only the energy losses associated with molecules 1–4 lie outside the 95% confidence interval as expressed by Linschoten et al.[23] for the reference compound *N*-isopropylphenoxypropanolamine.

The AM1 Hamiltonian[30] was used to estimate the stability of the planar molecule compared with the molecule where the para substituent was at 90° to the phenyl ring, and therefore unconjugated (see Experimental Section). If the wall of the β-1-adrenoceptor forces the para substituent of 1–7 out-of-plane with the phenyl ring, then one would expect the energy of conjugation to correlate with the value of "energy loss" (Table I, column IV). The semiempirical molecular orbital method AM1 was used in this study for the following reasons: (1) the method is well-tested and there are many examples in the literature where it has been used in the calculation of rotational barriers[31–33] and (2) reduction in the computation time when compared with ab initio methods. One shortcoming of the AM1 method is the reported tendency to underestimate the size of rotational barriers.[34,35] However, calculations using the ab initio STO-3G and 6-31G basis sets have previously been reported to overestimate the size of rotational barriers.[34,36]

The results are shown in Table I. By subtracting the difference in energy between the conjugated (column V) and nonconjugated forms (column VI) for molecules 1–5 an approximate value of the conjugation energy is obtained. There appears to be a correlation between the energy lost upon binding to the receptor (column IV) and the energy of conjugation (column VII). This supports our suggestion that, on binding, the para substituent is forced out-of-plane with the phenyl ring and this explains the lower binding energy for these molecules. Molecules 6 and 7 appear to be exceptions and an explanation for their biological activities is given later in the discussion.

For molecules 8–11, in Table I, conjugation between the para substituent and the phenyl ring would not occur. Instead, the most energetically stable side-chain conformation would be approximately 90° relative to the phenyl ring and this is reflected in the heats of formation shown in Table I. The rotational barrier for molecules 8, 9, and 11 is quite small, indicating that the para substituents would be able to rotate freely until bound to the receptor. There would then be no significant energy loss associated with orientation at 90° to the phenyl ring. In keeping with this argument, the discrepancy between the observed and calculated free energy of binding (i.e. energy loss Table I, column IV) for 8–11 is on average not as great as the values of molecules 1–5. Moreover, a consequence of our receptor wall theory (Figure 3), would be a loss of a degree of freedom between the aromatic ring and the para substituent for 8–11, which could be corrected by subtracting 3 kJ mol^{-1} from "energy loss".[37] This would reduce the discrepancy between the observed and calculated free energy for all this group of compounds except 8, which would bind 2 kJ mol^{-1} better than the reference compound (*N*-isopropylphenoxypropanolamine) after this adjustment.

In contrast to the above argument involving steric hindrance, there is no simple correlation ($r = 0.381$ for 10 data points; $p \gg 0.1$) between the hydrophobicity (log *P*, octanol/water) of the para substituent for molecules 1–5 and 7–11 and the activity at the β-1-adrenoceptor (given by pA_2(corr) in column II, Table I). However, when one compares close analogues 2 and 3, the difference in pA_2 values is 0.2 and the corresponding difference in hydrophobicity is 0.3. For molecules 4 and 7 the difference in pA_2 is 1.8, while the hydrophobicity difference is 2.4. Thus it appears that, at least in these two examples, as the hydrophobicity increases the activity at the β-1-adrenoceptor also increases. Hydrophobicity was estimated using the hydrophobic fragmental *f* values of Rekker and de

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Kort.[38] It may well be that the poor correlation between hydrophobicity and biological activity is due to the calculation method used to determine log P, and experimental log P values may show a different trend. This, however, was considered unlikely as the octanol/water system has been reported to be a rather poor model for drug interactions with biological membranes.[39-42] Model membranes such as dimyristoylphosphatidylcholine (DMPC)/physiological saline have been suggested as a more realistic system for estimation of hydrophobicity.[40-43] It is also possible that the reason for the poor correlation with log P is that it is only one of several factors determining binding energy and hence is more obvious when comparing close analogues (vide supra).

It has been proposed that the β-blocker's phenyl ring interacts with an aromatic residue, possibly tryptophan, in the receptor active site.[44-46] Therefore we examined whether there was a relationship between activity and the ability of the phenyl ring to interact with the active-site residue. Molecules 1–5 and 7, where the para substituent was in conjugation with the phenyl ring, had an overall ring charge which was more positive than those of molecules 8–11, in which such conjugation was absent. Apart from this general observation, there was no direct correlation between ring charge and activity ($r = 0.176$ for 10 data points; $p \gg 0.1$). There was no relationship for parameters such as dipole moment or the gap between HOMO and LUMO.

Preliminary studies on the molecular electrostatic potential (MEP) maps of these molecules show features which have been described in an earlier study on β-blocking agents.[47] Common to all molecules there exists a region of negative potential covering both the ortho and meta positions of the phenyl ring (referred to as center M2 in ref 47) as well as extending out approximately 1–2 Å from the para position (M3, ref 47). A positive potential region exists beyond the region of negative potential out from the para position (corresponding to the center P4 in ref 47). The MEP features described are observed regardless of the para substituent being in a planar conformation (torsion angle = 0°) or at 90° relative to the phenyl ring and do not appear to offer an explanation for the variation in binding energies.

Molecules 6 and 7 have greater biological activity than would be expected according to our model since both would be conjugated with the phenyl ring. The addition of a thiophene ring to the imidazole ring in molecule 5 results in 6, with a relative release of 8 kJ mol^{-1} of binding energy. Similarly, the addition of a (4-fluorophenyl)methyl group to molecule 4 results in 7, with a relative release of 11 kJ mol^{-1} of binding energy. One possible explanation of this improved binding would be the presence of a second binding site for these aromatic groups on the para substituent, and that this compensates for the energy required to break conjugation between the aromatic ring and the substituent. This would require that the fluorophenyl ring of molecule 7 could be superimposed upon the thiophene ring. (If they cannot be superimposed it would be unlikely that they could bind to the same second aromatic binding site in the receptor.) To test this hypothesis, we searched the conformational space available to 7 using distance and energy constraints in the SEARCH routine of SYBYL.[48] Only conformers which were within 80 kJ mol^{-1} of the energy for the conformer with the side chain in the extended form with the phenyl ring at 90° to the para substituent ($E = 29.7$ kJ mol^{-1}) were selected (see Experimental Section). Of the 19 conformers which resulted, the first two were selected for molecular mechanics minimization using MAXIMIN2.[48] Refined energies of 41.8 and 55.2 kJ mol^{-1} were obtained. The lower energy conformer ($E = 41.8$ kJ mol^{-1}) had an energy value 12.1 kJ mol^{-1} above that of the extended conformation and was considered to be realistically accessible. The rigid superimposition of molecule 7 on 6, using the normals of the appropriate aromatic rings of 6 as a template (root mean square over six atoms = 0.085), is shown in Figure 2. We suggest that an aromatic binding site could stabilize such a conformation in 7 and result in a reduced dissociation constant relative to 4. It must be stressed that the above conformational search has not necessarily found the lowest energy conformation for which 7 can fit onto 6, but has given support to the idea that such conformations are energetically feasible. Indeed, if 7 binds as in Figure 2, it suggests that the fluorophenyl ring must bind with an energy of approximately 23 kJ mol^{-1} in order to stabilize this conformer and yet still bind 11 kJ mol^{-1} better than 4. In support of this, the average expected[37] binding energy for a fluorophenyl ring is 23 kJ mol^{-1}.

To unite the above theories into a common model, we rigidly superimposed all 30 relevant structures (two conformers each of molecules 1–4 and 6, where the para substituent and phenyl ring are at 0° and 180° and which therefore constitute the "inactive" set, and two conformers each of 1–4 and 6–11, where the para substituent and phenyl ring are at 90° and 270° and which therefore constitute the "active" set), as shown in Figure 3. All molecules were aligned using the phenyl ring, apart from 7, which was superimposed on 6 as shown in Figure 2. For the sake of clarity the oxypropanolamine moiety has been replaced with a hydrogen atom. Using the MVOLUME routine in SYBYL,[48] we obtained the van der Waals volume which was common to all "inactive" conformers and substracted from this the total van der Waals volume of the complete "active" set. The resulting volume, which is

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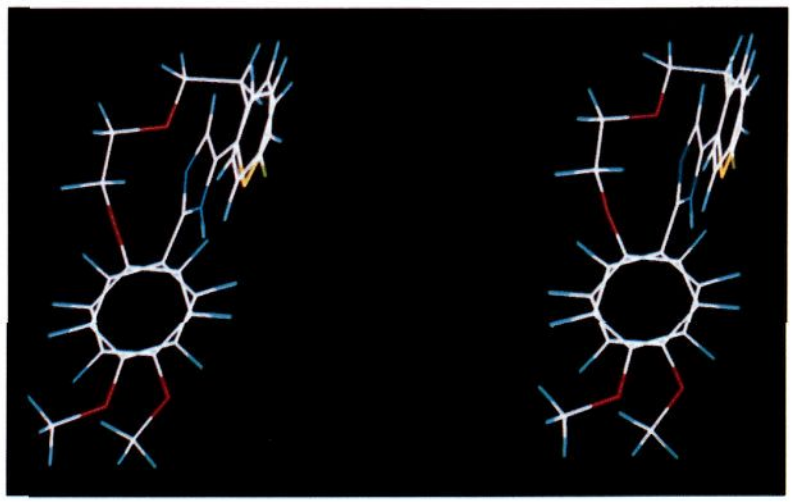

Figure 2. Relaxed stereoview of the superimposition of the two aromatic rings in **7** on the phenyl and thiophene rings of molecule **6**. The conformer of molecule **7** shown (E = 41.8 kJ mol^{-1}) is 12.1 kJ mol^{-1} higher in energy than that in which the alkyl side chain is extended. The propanolamine substructure has been replaced with a methyl group.

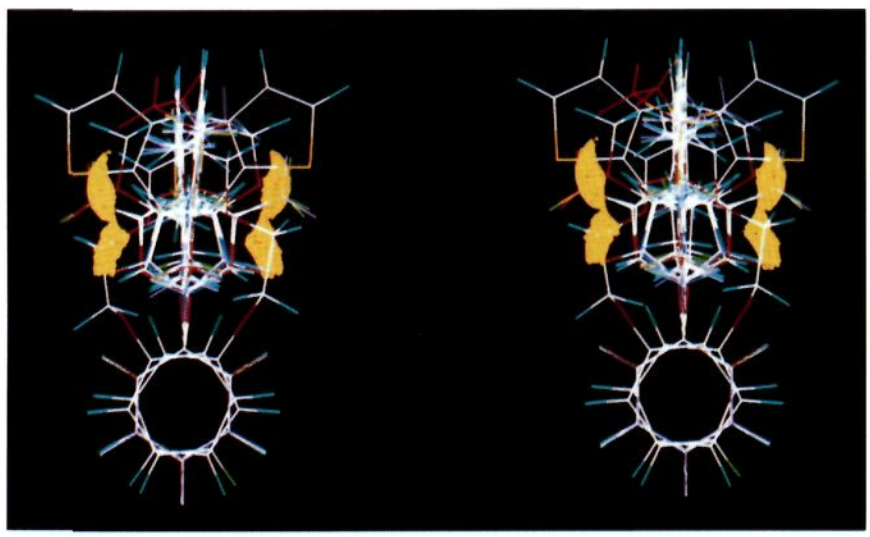

Figure 3. Relaxed stereoview of the van der Waals volume (yellow) which is common to all planar, low-affinity molecules **1–4** and **6**, where the phenyl ring and substituent are at 0° and 180° to each other. This volume is not shared by any of the "active" set of molecules **1–4** and **6–11** in which this angle is either 90° or 270° and could represent the wall of the β-1-adrenoceptor. The phenyl ring of each substituent was used for superimposition, except for molecule **7**, which was superimposed as shown in Figure 2. For the sake of clarity, the oxypropanolamine side chain has been replaced with a hydrogen atom.

Table II. Coordinates of Proposed Site Points[a] at the β-1-adrenoceptor

site point no.	description
13 (4.92, 3.38, −0.50)	wall of the β-1-receptor: "filled" site points
14 (0.61, 5.91, −0.44)	
15 (5.89, 4.98, −0.92)	
16 (1.55, 7.51, −0.86)	
17 (4.13, 7.00, −3.21)	aromatic binding sites[b]
18 (4.41, 7.39, 1.64)	

[a] Site points **1–12** were characterized by Linschoten et al.,[23] and the x, y, and z coordinates shown (angstroms) are referenced to these, with the assumption that the phenyl ring common to all molecules in Table I binds to site point **7**. [b] Due to the symmetrical nature of the molecules studied, either or both of these sites could exist.

shown in yellow in Figure 3, could represent the wall of the β-1-adrenoceptor.[49]

In Table II are shown the locations in space of the receptor wall and second aromatic binding site suggested in our study. The coordinates are referenced to Linschoten et al.'s[23] map of the β-1-adrenoceptor, assuming that the phenyl ring common to all these molecules binds to the site designated as point 7.

This study has concentrated only on compounds that have substituents in the para or 4 position of the phenyl ring. It has been shown that the introduction of small substituents at the 2-position of the phenyl ring in para-substituted compounds like practolol increases potency.[27] The barrier to rotation of the para substituent for molecules with either a 2-methyl or a 2-nitro group has been calculated using AM1 to be 14 kJ mol^{-1}. This is the same as the rotational barrier that has been calculated for practolol in this study. It therefore appears that the small 2-substituents have little influence on the preferred conformation of the para substituent (at least in the case of practolol). The increased potency of compounds containing both 2-substituents and para substituents could be explained by a favorable interaction between the receptor and the 2-substituent. The size of the 2-substituent seems to be an important factor, with larger substituents showing a reduction in potency.[27] This would

suggest a cavity in the receptor with size constraints, in which smaller groups (e.g. 2-NO_2, 2-F, 2-CH_3) can be accomodated. Compounds previously synthesized within our group containing both substituents in the 2 and 4 positions of the phenyl ring also exhibit this behavior.

Conclusion

The correlation between the calculated energies of conjugation for a number of para-substituted β-blockers and the observed β-1-adrenoceptor affinity suggests that in the act of binding of the receptor, the para substituent is forced out-of-plane with the phenyl ring by steric hindrance with the receptor wall. This fits the observation that a number of compounds in which the para substituent is not conjugated with the phenyl ring only exhibit a marginally lower binding energy than the reference compound *N*-isopropylphenoxypropanolamine and that this can be improved by correcting for loss of a degree of freedom. Hydrophobicity is known to have an affect on the potency of drugs, but in this study, no simple correlation existed between the hydrophobicity and receptor affinity.

We also suggest that there is an aromatic binding site near the thiophene ring of molecule **6**, also accessible to the fluorophenyl ring of **7**. These hypotheses were supported by the observation of a common volume occupied by the "inactive" structures, which was not occupied by any of the "active" structures and which could represent part of the β-1-adrenoceptor wall.[50] The location within the three-dimensional structure of the β-1-adrenoceptor of the receptor wall proposed in this study has yet to be elucidated.

Experimental Section

Biological Activity. pA_2 values for compounds **2**, **3**, **5**, **6**, **9**, and **11** were obtained from the literature.[25,50-52] Determination of pA_2 values for β-1-adrenoceptors for compounds **1** and **4**, **7**, **8**, and **10** were obtained from in vitro measurements of the ability to inhibit the dose–response curve to cumulative doses of isoprenaline in a guinea pig atrial preparation. Atria were equilibrated for 60 min with Krebs–Henseleit physiological salt solution at 37 °C gassed with carbogen (5% CO_2 in O_2), the composition of which was (mM) NaCl (118), KCl (4.7), $MgSO_4$ (1.2), $CaCl_2$ (2.5), KH_2PO_4, (1.2), $NaHCO_3$ (25), glucose (11.1), and EDTA (0.0025).

Cumulative concentration–response curves were obtained in each preparation as described by Van Rossum et al.[53] and fitted by computer analysis according to Zaborowsky et al.[54] For measurement of antagonist activity the appropriate agent was added to the organ bath at least 45 min after the first control concentration–response curve and allowed to equilibrate for 20 min before the next concentration–response curve was established. The shift in this curve to the right was calculated as a pA_2 value.[55] Control curves repeated in the absence of antagonists showed no significant shift.

Standardization of pA_2 values. The pA_2's of all molecules were normalized relative to the pA_2 for practolol reported by Bilezikian et al.[56] The difference between the pA_2 of practolol obtained by a given group with that reported by Bilezikian et al.,[56] whose data formed the basis of Linschoten's et al.'s[23] receptor mapping work, was subtracted from the pA_2 of any other molecules reported by that group after adjusting the pA_2's for the proportion of *S*-isomer. It is this enantiomer which overwhelmingly accounts for the biological activity in phenoxypropranolamine-based β-blockers.[57] For instance, Bilezikian et al.[56] reported a log K_d for racemic practolol on the turkey erythrocyte β-1-receptor of −5.01. To obtain the log K_d of the binding species, Linschoten et al.[23] adjusted this figure to −5.31 after consideration of the proportion (50%) of *S*-enantiomer. Baldwin et al.[50,51] however, reported a pA_2 of 6.98 for practolol on guinea pig atria, which becomes 7.28 after adjustment for the *S*-enantiomer and 5.31 after standardization. Thus to derive the corrected pA_2 values (pA_2(corr), column II in Table I) for molecules **5** and **6**, which came from Baldwin et al.'s[50,51] study, we adjusted the published pA_2 values (pA_2, column I in Table I) for the amount of *S*-enantiomer by adding 0.3, and then subtracted 1.97 (=7.28 − 5.3).

Correction for the proportion of cation, which is throught to be the active species,[58] using the method of IJzerman et al.[58,59] was not made, since at physiological pH these drugs are almost entirely ionized and correlations are negligible. pA_2 values for racemic practolol of 6.0, 6.6, and 6.5 were obtained by us, by Ehrardt et al.[52] and by Erez et al.,[25] respectively. These led to corrections of −1.0, −1.6, and −1.5, respectively, to the pA_2's of any molecules reported by the above workers after each had been adjusted for the amount of *S*-enantiomer (by adding 0.3 if a racemic mixture was tested).

Calculation of Free Energy of Binding. After normalization of the pA_2 values (see above paragraph), the observed Gibbs free energy of binding ($\Delta G°$, kJ mol^{-1}) was obtained from the reported pA_2's or log K_d's using the equations

$$p_{A2} = -\log K_d \text{ (ref 60)} \quad (1)$$

$$\Delta G° = -RT \ln K_a \quad (2)$$

$$= RT \ln K_d, \quad (3)$$

$$= 5.96 \log K_d \quad (4)$$

and physiological conditions (37 °C).

Materials. The following compounds were obtained: (±)-practolol (ICI), (±)-H87/07 (Astra), (±)-Ro-31-1118 (Roch), (±)-betaxolol (Synthelabo), and (±)-atenolol (Sigma).

Computer Modeling. All minimizations were based on in vacuo conditions and all modeling was performed on a Silicon Graphics Personal IRIS using the molecular modeling software package SYBYL[48] (version 5.32). All molecular mechanics

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calculations used MAXIMIN2 with the Tripos force field[61] and took into account electrostatics. The charges on molecules were calculated using the GAST-HUCK algorithm, which incorporates Gasteiger–Marsili[62] and Huckel[63] calculations. Molecules were deemed to be minimized when there was a minimum energy change of less than 0.21 kJ mol^{-1} for one iteration. The conjugate gradient method was used for minimization. All AM1 calculations involved the singlet state. Molecules were deemed minimized when the gradient fell to less than 0.04 kJ mol^{-1} or when SELCON fell to less than 4.2×10^{-7} kJ mol^{-1}. These are the values imposed when the keyword "PRECISE" is specified. Molecules were built up using the SYBYL molecular fragment library.[48] To save computing time, the propanolamine side chain was assumed to have negligible effect on either the degree of conjugation between the aromatic ring with the para substituent or the preferred conformations of the para substituent and was replaced with a methyl group, thereby rendering all compounds methoxyphenyl (anisole) derivatives. For the calculation of energy of conjugation, molecules 1–5 were minimized in their planar conformations, with the side chains in the energetically preferred trans (molecules 1–3) or extended (molecule 4) conformations, using initially the molecular mechanics algorithm MAXIMIN2.[48] The molecules were then fully minimized, using AM1 (MOPAC version 5.0), to a level 100 times more refined than that of the default by specifying the keyword "PRECISE". Herbert's test was satisfied in BFGS, and the SCF field was achieved for all molecules. To obtain the heats of formation corresponding to the unconjugated conformers the torsion angle between the phenyl ring and the substituent was altered to 90°, and all geometrical parameters except this torsion angle were minimized. For the conformational search of molecule 7, the distance constraints imposed required the terminal dummy atoms of the normals (each of which was 2 Å long) of the phenyl and fluorophenyl ring to be within ±0.1 Å of those of the phenyl and thiophene ring, respectively, of molecule 6. All seven rotatable torsion angles of molecule 7 were explored in 30° steps from 0 to 360°, except for τ_1, which was constrained to 80–100° and 260–280° and searched in 10° steps, and τ_2, which was searched from 0 to 360° in 10° steps (both τ_1 and τ_2 are defined in Table I).

The SYBYL routine MVOLUME was used to calculate and display the van der Waals volume contoured in all planes and calculated on a grid of 0.15 Å.

Hydrophobicity Calculations. The log P values for the para substituents given in Table I (column VIII) were calculated using the hydrophobic fragmental system of Rekker and de Kort.[38]

Acknowledgment. We appreciate helpful comments from Dr. Margaret Wong and the typing of the manuscript by Miss Allison Quinn. The work was supported by a program grant from the NH&MRC.

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