Adrenoceptor Blocking Agents. Compounds Related to Metoprolol

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A group of compounds, structurally related to metoprolol, in which the aromatic nucleus is formally moved stepwise away from the ethanolamine side chain, has been studied as adrenergic agonists and antagonists. All the compounds were active on the adrenergic receptors and showed similar affinity for the receptor regardless of the distance between the aromatic nucleus and the ethanolamine moiety. An ethereal oxygen may be of importance for the affinity to the receptor but this oxygen may not necessarily have to be located as an OCH_2 group between the aromatic ring and the ethanolamine chain of the β-blocker molecule.

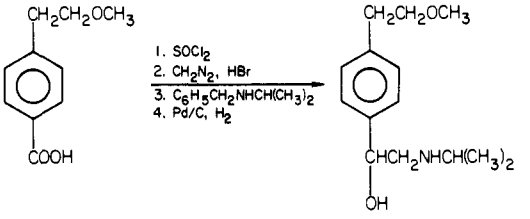
β-Adrenergic receptor antagonists may be of the arylethanolamine type (e.g., pronethalol or sotalol) or the (aryloxy)propanolamine type (e.g., propranolol or metoprolol). The majority of the β-blockers in clinical use today are of the latter type, and it is considered that this type of compound generally is more potent than the arylethanolamine analogues.

Arylethanolamines or (aryloxy)propanolamines may also be adrenergic agonists. In a previous study on benzofuranylethanolamines we found that 1-(5-hydroxy-2-benzofuranyl)-2-(isopropylamino)ethanol is a β_1-selective adrenergic agonist with high intrinsic activity.[1] Comparing this compound with for example, isoprenaline, it was proposed that the aromatic moiety of arylethanolamines and (aryloxy)propanolamines may bind to different functional groups of the receptor. We have now investigated this possibility further for some adrenergic antagonists.

Since the discovery of propranolol and related (aryloxy)propanolamines, most investigators of new β-blockers have studied only this class of compounds. The adrenergic receptor can bind and be activated by both the arylethanolamines and the (aryloxy)propanolamines, although the aromatic ring is separated from the ethanolamine moiety by two atoms in the latter type of compounds. In spite of this observation, no systematic study of the adrenergic receptor affinity in relation to the distance between the ethanolamine chain and the aromatic ring has been performed. Since we considered such a study to be of interest for a better understanding of adrenergic agonists and antagonists, we have investigated the phenylethanolamine analogue of metoprolol[2] (**2**) and a group of related compounds where the aromatic nucleus is located at different distances from the ethanolamine moiety. Four of the compounds are chemical isomers and thus have very similar lipophilic properties. Differences in receptor affinity are therefore likely to be due to the location of the aromatic ring in relation to the ethanolamine moiety, which is kept constant in all the molecules. The compounds and their biological activities are presented in Table I.

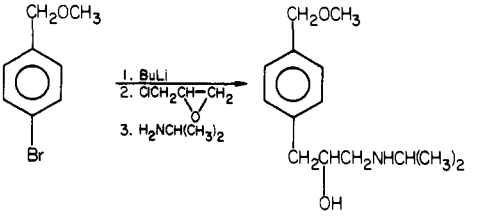
Chemistry. Compound **2** was prepared as depicted in Scheme I. 4-(2-Methoxyethyl)benzoyl chloride was converted to the corresponding bromo ketone in one step using CH_2N_2 and HBr. Treatment of this product with benzylisopropylamine, followed by hydrogenation using Pd/C as catalyst, yielded the ethanolamine, **2**.

Scheme I



2

Scheme II



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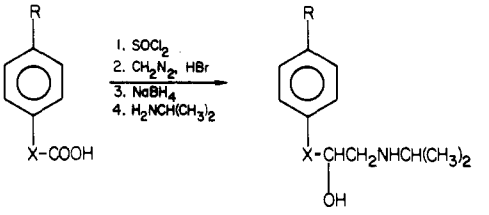
Scheme III



4, R = OCH_3; X = CH_2CH_2
5, R = CH_3; X = OCH_2CH_2

Substance **3** was obtained from 1-bromo-4-(methoxymethyl)benzene by treating it with BuLi and subsequently with epichlorohydrin as shown in Scheme II. Alkylation with isopropylamine yielded compound **3**. Compounds **4** and **5** were prepared as illustrated in Scheme III. The appropriate acid chloride was converted to the corresponding bromo ketone as described above. This was then reduced using $NaBH_4$, and the product was reacted with isopropylamine to yield the desired compounds.

Pharmacology. The affinity and the intrinsic activity of the compounds were determined using a pharmacological model offering one β_1- and another β_2-mediated effector. Heart rate and peripheral vascular resistance in the reserpinized, vagotomized cat were chosen as the β_1- and β_2-mediated variables, respectively, and the compounds were tested as described under Experimental Section. The K_B values for antagonistic effect on heart rate and peripheral vascular resistance, the intrinsic ac-

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Table I. Structures and Biological Properties of the Compounds Studied

no.	compd	mp, °C	formula[b]	n[c]	effects on heart rate: weighed K_B ± SD, μmol/kg	effects on heart rate: intrinsic act., % of HR max at IPR	effects on heart rate: geom mean K_A ± SD, μmol/kg	effects on peripheral vascular resistance: weighed K_B ± SD, μmol/kg
1	$CH_3OCH_2CH_2-Ph-OCH_2CH(OH)CH_2NH-$ · HCl[a] (Metoprolol)			5	0.084 ± 0.055			0.87 ± 1.17
2	$CH_3OCH_2CH_2-Ph-CH(OH)CH_2NH-$ · HCl	101–102	$C_{14}H_{24}ClNO_2$	4	3.22 ± 2.79	22.5 ± 2.1	0.536 (0.111–2.57)	23.60 ± 42.15
3	$CH_3OCH_2-Ph-CH_2CH(OH)CH_2NH-$ · HCl	119–121	$C_{14}H_{24}ClNO_2$	4	5.83 ± 13.06			6.10 ± 6.64
4	$CH_3O-Ph-CH_2CH_2CH(OH)CH_2NH-$ · HCl	152–154	$C_{14}H_{24}ClNO_2$	4	3.96 ± 3.02	4.5 ± 3.7		30.80 ± 32.63
5	$CH_3-Ph-OCH_2CH_2CH(OH)CH_2NH-$ · HCl	144–146	$C_{14}H_{24}ClNO_2$	4	17.10 ± 12.22			1.82 ± 2.43
6	$CH_3O-Ph-OCH_2CH(OH)CH_2NH-$ · HCl	142–143	$C_{13}H_{22}ClNO_3$	4	1.03 ± 1.01	81.2 ± 5.2	0.119 (0.075–0.188)	3.42 ± 3.60

[a] See ref 2. [b] Compounds analyzed for C, H, and N. [c] Number of animals used.

tivities, and the K_A values for agonistic effects on heart rate are given in Table I.

Results and Discussion

Metoprolol (1) is a β_1-selective adrenergic antagonist with a K_B value (heart rate) of 0.08 μmol/kg. Its phenylethanolamine analogue 2 is also an antagonist with comparable selectivity, although its affinity for β_1 and β_2 receptors is about 30 times lower than that of metoprolol. This is in agreement with the general notion that the oxypropanolamines are more potent than their ethanolamine analogues. In contrast to metoprolol, 2 also has an intrinsic activity of 23% of that of isoprenaline. For receptor agonists and antagonists of type 2, it can be assumed that binding of the nitrogen and the oxygen of the ethanolamine moiety, as well as of the aromatic ring, to appropriate functional sites is important for activation and/or blockade of the β-adrenergic receptor.[3,4] The adrenergic agonist prenalterol[5] and the previously mentioned 1-(5-hydroxy-2-benzofuranyl)-2-(isopropylamino)ethanol are both of the (aryloxy)propanolamine type. If the nitrogen and the oxygen of such compounds bind to the receptor in the same manner as the arylethanolamine-type compounds, the aromatic nucleus of the two types of compounds cannot be brought to the same spatial position. It was therefore suggested that the aromatic moieties of arylethanolamines and (aryloxy)propanolamines bind to different functional groups of the receptor.[1]

Compounds 2–5 are all β-adrenergic antagonists, although less active than metoprolol. They are also chemical isomers with very similar lipophilic properties. Formally, the aromatic nucleus is stepwise moved away from the ethanolamine side chain, thus offering an opportunity to study the structural requirements of the receptor regarding the distance between the aromatic ring and the ethanolamine moiety without any major effects on other properties of the compounds.

Compounds 3 and 4 have approximately the same affinity for the β_1 receptor as their isomer 2, whereas compound 5 is 4–5 times less active. Two of the isomers, 2 and 4, have a considerably lower affinity for the β_2 than for the β_1 receptor, whereas 3 has equal affinity for both types of receptors. It is an unexpected observation that 2–4 all have approximately the same affinity for the β_1 receptor. In compound 3 the phenyl group is bound directly to the propanolamine side chain, whereas 4 is a methylene homologue of the (aryloxy)propanolamine-type compounds.

When the phenyl group is moved another step away from the ethanolamine moiety, as in compound 5, the molecule is still as potent as isomers 2–4. However, its selectivity in β blockade is now opposite that of 2 and 4, from the cardiac to the vascular receptors. Compound 5 may thus be considered a selective β_2 antagonist with a K_B (HR)/K_B (PR) ≈ 10. It should also be noted that the affinity of 5 for the β_2 receptor is 17 times greater than that of 4.

A comparison of the affinity data for compounds 2–5 indicates that the ethanolamine moiety and the phenyl ring may be separated by 0, 1, 2, or 3 atoms and the compounds still have approximately the same affinity for the β receptors, although the individual affinities for the β_1 and the β_2 receptors differ. It can thus be concluded that the

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distance between the Ar group and the ethanolamine chain is not very critical for β blockers. Similar observations have recently been published by Leclerc et al.[6] who found that for certain oxime ethers it was possible to move the aromatic ring away from the ethanolamine chain and also to replace it by an alkyl moiety without affecting the potency or affinity of these β-adrenoceptor blocking agents.

(Aryloxy)propanolamines are generally considered to be more potent β blockers than compounds of type 4, where the ethereal oxygen is replaced by a CH_2 group.[7] We were therefore interested in comparing compound 4 with the corresponding hydroquinone derivative 6. Our results indicate that the latter compound has an affinity for β_1 and β_2 receptors that is only 4 and 10 times, respectively, that of compound 4. In addition, 6 has a high intrinsic activity comparable to that of prenalterol, which also has oxygens directly bound to the aromatic ring in positions 1 and 4. No β_2-adrenoceptor agonistic effect was observed with this or with the other compounds studied.

Howe[8] found that replacement of the ethereal oxygen of propranolol by a methylene group reduced the potency to less than 0.01 of that of the parent compound and suggested that the ethereal oxygen of (aryloxy)propanolamines is involved in the receptor binding via its unshared electrons. In their recent study, Leclerc et al.[6] obtained inactive compounds by replacing an ethereal oxygen by a nitrogen in certain nonaromatic β blockers and, therefore, concluded that an ethereal oxygen seems to be crucial for β-adrenergic activity. Our data do not support the conclusions of these two reports, since we found that compounds 4 and 6 have affinities of the same magnitude.

All compounds presented here have an ethereal oxygen in the molecule either in the aliphatic side chain, as in 2 and 3, or directly bound to the aromatic ring, as in 4 and 5. A corresponding oxygen is not present in the methylene analogue of propranolol. This difference may indicate that an ethereal oxygen is important for the affinity but it may not necessarily have to be located as an OCH_2 group between the aromatic ring and the ethanolamine chain.

Experimental Section

Melting points were determined on a Mettler FP1 apparatus. IR spectra were measured on a Perkin-Elmer 337 spectrophotometer. NMR spectra were taken on a Varian T-60 or a Brucker WH 270 instrument with $CDCl_3$ solutions containing Me_4Si as an internal standard. Mass spectra were recorded with an LKB 9000 instrument at 70 eV. Reaction products were checked routinely by IR, NMR, and mass spectrometry. All compounds showed the expected spectral characteristics. Elemental analyses were performed by the Microanalytical Laboratory at the Institute for Agriculture, Uppsala. The analytical results obtained were within ±0.4% of the theoretical values.

4-(2-Methoxyethyl)phenyl (*N*-Benzyl-*N*-isopropylamino)methyl Ketone. A solution of 18.0 g (0.1 mol) of 4-(2-methoxyethyl)benzoic acid,[9] 21.4 g (0.18 mol) of $SOCl_2$, and 200 mL of $CHCl_3$ was heated under reflux for 3 h. The solution was cooled, and the $CHCl_3$ and excess $SOCl_2$ were evaporated. An ether solution of 10.0 g (0.05 mol) of the residue was carefully added to 4.2 g (0.1 mol) of CH_2N_2 in ether and the reaction mixture was stirred for 30 min, whereupon 20 mL of HBr (48%), 3 mL of H_2O, and 20 mL of ether were added dropwise at 0 °C. The mixture was stirred at 0 °C for 1 h and at room temperature for 3 h, and 100 mL of H_2O was then added. After separation, the ether phase was washed successively with saturated $NaHCO_3$ solution, H_2O, and saturated NaCl solution. The dried (Na_2SO_4) solution was evaporated, and the bromomethyl ketone (10.5 g) was refluxed without further purification with 15.0 g of benzylisopropylamine in 150 mL of 2-propanol for 3 h. Benzylisopropylamine hydrochloride precipitated when the reaction mixture was cooled. After filtration and evaporation, the residue was dissolved in 2-propanol and HCl/ether was added, yielding 9.8 g (67%, calculated on the amount of crude bromomethyl ketone) of the title compound as the HCl salt, mp 131–132 °C (from 2-propanol/ethanol). Anal. ($C_{21}H_{27}NO_2$·HCl) C, H, N.

(±)-2-(Isopropylamino)-1-[4-(2-methoxyethyl)phenyl]ethanol (2). The above-named aminomethyl ketone hydrochloride (9.2 g, 0.0255 mol) was hydrogenated in absolute EtOH with Pd/C as catalyst. After absorption of a precalculated amount of hydrogen, the solution was filtered, the solvent was evaporated, and the product was recrystallized from EtOH/EtOAc: yield 6.0 g (86%); mp 101–102 °C. Anal. ($C_{14}H_{23}NO_2$·HCl) C, H, N.

(±)-1-(Isopropylamino)-3-[4-(methoxymethyl)phenyl]-2-propanol (3). 1-Bromo-4-(methoxymethyl)benzene[10] (19.8 g, 0.098 mol) was dissolved in 50 mL of ether, and 50 mL of a 2 M solution of *n*-butyllithium (0.1 mol) was added over a period of 10 min. The reaction mixture was then stirred for 30 min at 20 °C. Epichlorohydrin (9.25 g, 0.1 mol) was dissolved in 50 mL of ether and, while the solution was cooled to −78 °C, the ether–hexane solution with the lithium reagent was added over a 30-min period. The resulting mixture was stirred at −78 °C for 1.5 h and was then allowed to warm up to room temperature. The reaction mixture was hydrolyzed using 2 M H_2SO_4, and the layers were separated. The ether layer was washed with water, Na_2CO_3, water, and aqueous NaCl and dried (Na_2SO_4) before evaporation. Due to decomposition, the residue could not be distilled. The crude reaction product was therefore dissolved in 30 mL of 2-propanol, isopropylamine (12 g, 0.2 mol) was added, and the solution was refluxed for 3 h. After evaporation, 2 N HCl was added, the solution was extracted with ether, and the extract was discarded. The aqueous layer was then made alkaline using 10 N NaOH and again extracted with ether. The ether extract was dried (Na_2SO_4) and concentrated, and saturated ethereal HCl gas was added. The HCl salt of 3 was collected by filtration: yield 4.1 g (15%); mp 117–121 °C (from EtOH). Anal. ($C_{14}H_{23}NO_2$·HCl) C, H, N.

(±)-1-(Isopropylamino)-4-(4-methoxyphenyl)-2-butanol (4). 3-(4-Methoxyphenyl)propanoic acid[11] (0.1 mol) was reacted with $SOCl_2$ and CH_2N_2 as described above for the preparation of compound 2. Sodium borohydride (6.3 g) was then added portionwise to a stirred solution of the bromomethyl ketone (14.3 g, 0.056 mol) dissolved in 200 mL of MeOH at 0 °C. The reaction mixture was stirred at 20 °C for 5 h, whereupon the solvent was evaporated, 30 mL of 2 N HCl was added to the residue, and the mixture was extracted with chloroform. The combined extracts were washed with water, dried (Na_2SO_4), and evaporated to give 11.4 g of the bromohydrin as an oil. The crude product (6.8 g, 0.026 mol) was refluxed with isopropylamine (4.7 g, 0.08 mol) in 100 mL of 2-propanol for 18 h. The solvent was then evaporated, and the residual oil was dissolved in 2 N HCl and extracted with ether. The acidic aqueous solution was made alkaline with 10 N NaOH and then again extracted with ether. This ether extract was dried (Na_2SO_4) and the solvent evaporated, yielding a solid residue which was recrystallized from chloroform/toluene: yield 3.1 g (50%); mp 138–139 °C; mp (HCl salt) 152–154 °C (from MeOH/ether). Anal. ($C_{14}H_{23}NO_2$·HCl) C, H, N.

(±)-4-(Isopropylamino)-1-(4-methylphenoxy)-3-butanol (5). This compound was prepared from 3-(4-methylphenoxy)propanoic acid[12] as described for 4: yield 2.7 g (44%); mp (HCl salt) 144–146 °C (from MeOH/ether). Anal. ($C_{14}H_{23}NO_2$·HCl) C, H, N.

(±)-1-(4-Methoxyphenoxy)-3-(isopropylamino)-2-propanol (6). 1-(4-Methoxyphenoxy)propylene oxide (73.4 g, 0.41 mol) was heated in a sealed vessel at 100 °C for 2 h together with 40 mL of isopropylamine and 150 mL of absolute ethanol. The product was treated as described for 3 and isolated as the HCl salt: yield 51.7 g (46%); mp 143 °C (from 2-propanol), lit.[13] 141 °C. Anal. ($C_{13}H_{21}NO_3$·HCl) C, H, N.

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Pharmacology. Cats of both sexes, weighing between 2.6 and 3.2 kg, were starved for 24 h and pretreated with reserpine (Serpasil, 2.5 mg/mL), 5 mg/kg of body weight, 18 h before experimentation in order to provoke neuronal noradrenaline depletion, thereby inducing a chemical sympathetectomy. The animals were anesthetized by a pentobarbital (Mebumal, 60 mg/mL ACO) injection, 30 mg/kg of body weight, intraperitoneally and maintained at a constant level of anesthesia by a continuous infusion of pentobarbital sodium (Mebumal), 0.1 mg min^{-1} (kg of body weight).$^{-1}$

Artificial respiration was given by means of a respiratory pump (Braun) connected to a tracheal cannula.

The cats were vagotomized bilaterally and the right carotid artery was catheterized for recording of the mean arterial blood pressure via a Statham P 23 strain gage pressure transducer. The heart rate was recorded via a Grass 7P4 cardiotachograph, which was triggered by the blood-pressure oscillations.

The pharmacological effects on the peripheral vascular resistance in one hind leg were estimated as follows: A plastic catheter loop was introduced into the left femoral artery in the inguinal region and connected to a peristaltic pump (Watson Marlow) which delivered a constant flow of >10 mL/min. The hind leg was thus perfused with the cat's own blood at a constant flow rate. Alterations in vascular resistance measured as changes in perfusion pressure were recorded via a Statham P 23 strain gage pressure transducer connected to the loop distal to the roller pump.

The left jugular and right femoral veins were catheterized for barbiturate and test compound administration, respectively.

All recordings were made on a Grass 7D polygraph. The blood gas status and hematocrit values of the animal were controlled continuously throughout the experiment and were regarded as normal within the following ranges: pH, 7.38 ± 0.06; pCO_2, 4.1 ± 0.2 kPa; HCO_3, 19.0 ± 1.0 mequiv/L; hematocrit, 30 ± 10%.

A standard fluid therapy (Ringer acetate, ACO, 20 mL/kg of body weight, + Macrodex, Pharmacia, 5 mL/kg of body weight, infused at 0.5 mL/min) was used to compensate for the fluid loss due to the reserpine pretreatment.

Maximal heart rate and peripheral vascular effects were obtained by iv and ia injection of supramaximal doses of isoprenaline (IPR).

The test compounds were infused iv for 10 min at a rate of 1.0 mL/min in five stepwise raised concentrations. Each infusion was followed by an iv and an ia injection of IPR in concentrations which in the absence of test compounds induced control responses equal to about 80% of the maximal IPR effects.

Knowing that the submaximal IPR concentration used induces a response which may be identified at the top of the linear part of the IPR dose–response graph and assuming that any antagonistic property of the test compound represents a perfectly competitive interaction with IPR at the receptor site, any decrease in the response to the IPR standard concentration will reflect a parallel rightward shift of the IPR dose–response curve.

Dissociation constants characteristic of the affinity for the receptors were calculated for agonistic (K_A) and antagonistic (K_B) effects of the test compounds.

The doses of the test compounds producing semimaximal excitatory effects on the heart rate were taken as estimates of K_A. The K_B values for antagonistic effects on heart rate and peripheral vascular dilatation were calculated according to Åblad et al.[14]

The formula used provided us with a series of K_B values, one for each dose of test compound, in every experiment. These values, which theoretically should be identical with each other within one experiment, were, however, widely scattered due to a possible nonlinearity of the dose–response curve segment at its extreme points (i.e., around the 20–30% and the 70–80% levels).

For calculation of the "mean" K_B of each experiment, a "weighing" formula[14] was used.

Acknowledgment. We thank Drs. Peder Berntsson and Arne Brändström for valuable discussions and Hans Thorin for the mass spectra.

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Notes

Structure-Activity Relationships in Dihydropteroate Synthase Inhibition by Sulfanilamides. Comparison with the Antibacterial Activity

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A set of 12 acidic, 5 imidic, and 5 amidic sulfanilamides (SA) were tested for their inhibitory activity on dihydropteroate synthase of *Escherichia coli*. The enzyme inhibition indexes (EII_{50}) were compared with the growth inhibition indexes (GII_{50}), and electronic structures of SA and cell permeability effects were discussed as possible determinant factors of the observed variation of the activity in the SA set. The results strongly support the following conclusion: (a) permeability factors are highly effective in depressing the activity of SA in growth inhibition with respect to enzyme inhibition, but they do not appear to contribute significantly to the activity variation; (b) the activities of the different SA, both in growth and enzyme inhibition experiments, are well accounted for by the electronic features of these compounds.

Studies[1-3] on the synthesis of dihydropteroate and folate in several bacterial species, and particularly in *E. coli*, have definitively shown that sulfanilamides (SA) carry out their antibacterial action by inhibiting, competitively with respect to *p*-aminobenzoate, the enzyme dihydropteroate

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