Notes

Cardioselectivity of β-Adrenoceptor Blocking Agents. 1. 1-[(4-Hydroxyphenethyl)amino]-3-(aryloxy)propan-2-ols

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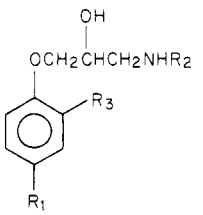
Received October 30, 1978

A series of 1-[(4-hydroxyphenethyl)amino]-3-(aryloxy)propan-2-ols was synthesized together with several 1-[(3,4-dimethoxyphenethyl)amino]-3-(aryloxy)propan-2-ols. Their affinity to β_1- and β_2-adrenoceptors was determined and compared with the affinity of known β-blockers. We were able to confirm the substantial cardioselectivity of 1-(3,4-dimethoxyphenethyl)-3-[(4-substituted aryl)oxy]propan-2-ols when compared to those with a 1-(4-hydroxyphenethyl) group. An increase in the size of the 4 substituent of the 3-(aryloxy) moiety to caproamido leads to a substantially higher affinity for the β_1-adrenoceptor of rat ventricular muscle in the presence of the 3,4-dimethoxyphenethyl than in the presence of the 4-hydroxyphenethyl or isopropyl group; this combination also gave the highest cardioselectivity.

The cardioselectivity of β-adrenoceptor blockers depends on a large variety of properties. Based on the classification of Lands et al.,[1] we expect that a cardioselective β-adrenoceptor blocker would show at the molecular level a higher affinity (as measured by the apparent dissociation constant) to β_1- than to β_2-adrenoceptors.[2,3] In vivo cardioselectivity, as measured by the physiological responses, may or may not correlate with the affinity measured at the molecular level. The disparity may be due to the blood clearance, metabolism, and distribution.

The discovery of practolol[4] began the search for β-adrenoceptor blockers of higher affinity and selectivity toward the β_1-adrenoceptor. Significant enhancement of cardioselectivity may be achieved by the placement of (1) a polar substituent R_1 in the 4 position of the 3-(aryloxy) group of 1-[(arylalkyl or alkyl)amino]-3-(aryloxy)-propan-2-ol (I),[5-8] (2) certain arylalkyl or alkyl groups, R_2,



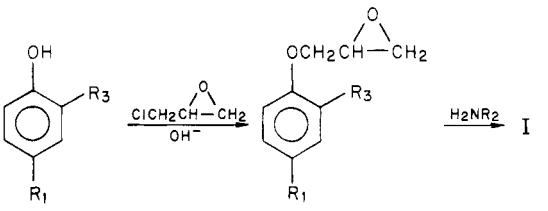
I

on the amino group,[9-11] or (3) 1-methyl substitution in the propan-2-ol moiety.[12] Thus, skillful simultaneous manipulation of R_1 and R_2 or R_3 and the side-chain substitution seems to give the highest available cardioselectivity.

In order to complement the existing evidence and further explain the structural requirements vesting cardioselectivity and high affinity, we describe herein the synthesis of several β-adrenoceptor blockers and the determination of their apparent dissociation constants.

Chemistry. As illustrated in Scheme I, the phenol substrates II (purchased or synthesized by well-known methods) were converted to epoxide intermediates using the conditions described by Shtacher.[13] The epoxides were purified by crystallization from ethyl acetate or column chromatography on silica gel using 10% MeOH in CH_2Cl_2. The reaction of the epoxides III with an excess of amine (50-fold for isopropylamine; 1.4-fold for 3,4-dimethoxyphenethylamine or 4-hydroxyphenethylamine) in boiling

Scheme I



II III

methanol gave the desired product I. The purification of the 3,4-dimethoxyphenethyl and 4-hydroxyphenethyl compounds often required repeated preparative LC. Synthesized compounds are listed in Table I.

Pharmacological Results and Discussion. The apparent dissociation constants (K_{app}) of the β-adrenoceptor blockers used in this study were determined using a competitive binding assay with [1-^{3}H]dihydroalprenolol ([^{3}H]DHA). All K_{app} were determined to a coefficient of variation ≤0.5. The preparation of rat ventricular muscle (RVM) and lung (RLM) has been previously described in detail.[2]

The aim of our investigation is to elucidate the nature of the influence of the 1-[(arylalkyl)amino] group (R_2) and the 4 substituent (R_1) on the aryloxy group of I on the cardioselectivity and affinity to the β_1-adrenoceptor. Selection of the 4-hydroxyphenethyl moiety was based on the structural similarity to the cardioselectivity-vesting 3,4-dimethoxyphenethyl group and the ease of preparation of potential radioiodinated diagnostic agents.[15] Compounds **1–3** (Table II) were considered the base compounds because of the lack of substitution of the 3-(aryloxy) group. The replacement of isopropyl in 1 with 3,4-dimethoxyphenethyl (2) results in a modest increase in cardioselectivity. The affinity to the β_1-receptor (RVM) remains the same with an apparent lowering of the affinity to the β_2-receptor (RLM). Introduction of 4-hydroxyphenethyl (3) decreases the cardioselectivity compared to **1**; this substituent does not alter the affinity to the RLM receptor but leads to a decrease in affinity to RVM (1 vs. 3).

Hoefle et al.[9] reported a substantial increase in cardioselectivity upon replacement of the isopropyl group with the 3,4-dimethoxyphenethyl group in the practolol molecule. In our study, using RVM and RLM, the increase

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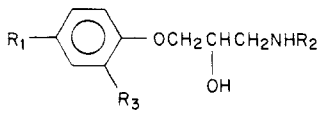
Table I. Chemical Data



compd	R_1	R_2	R_3	mp, °C	yield %[a]	recrystn solv	formula[b]
1[c]	H	$CH(CH_3)_2$	H	144-146	47	MeOH	$C_{12}H_{19}NO_3 \cdot C_2H_2O_4$[d]
2[e]	H	$3,4\text{-}(CH_3O)_2C_6H_3CH_2CH_2$	H	156-158	27	MeOH	$C_{19}H_{25}NO_4 \cdot C_2H_2O_4$
3	H	$4\text{-}HOC_6H_4CH_2CH_2$	H	202-204	12	MeOH	$C_{17}H_{21}NO_3 \cdot C_2H_2O_4$
4[d]	CH_3CONH	$3,4\text{-}(CH_3O)_2C_6H_3CH_2CH_2$	H	218-220	63	EtOH	$C_{21}H_{28}N_2O_5 \cdot C_2H_2O_4$
5	CH_3CONH	$4\text{-}HOC_6H_4CH_2CH_2$	H	183-185	18	MeOH	$C_{19}H_{25}N_2O_4 \cdot C_2H_2O_4$
6[f]	$CH_3(CH_2)_4CONH$	$CH(CH_3)_2$	H	131-133	16	MeOH	$C_{18}H_{30}N_2O_2 \cdot C_2H_2O_4$
7	$CH_3(CH_2)_4CONH$	$3,4\text{-}(CH_3O)_2C_6H_3CH_2CH_2$	H	167-170	17	EtOH	$C_{25}H_{36}N_2O_5 \cdot C_2H_2O_4$
8	$CH_3(CH_2)_4CONH$	$4\text{-}HOC_6H_4CH_2CH_2$	H	201-203	47	H_2O	$C_{23}H_{32}N_2O_4 \cdot C_2H_2O_4$
9	$CH_3(CH_2)_4CONH$	$3,4\text{-}(CH_3O)C_6H_3CH_2CH_2$	Cl	145-147	55	EtOH	$C_{25}H_{35}ClN_2O_5 \cdot C_2H_2O_4$
10	$CH_3(CH_2)_4CONH$	$4\text{-}HOC_6H_4CH_2CH_2$	Cl	181-183	41	MeOH	$C_{23}H_{31}ClN_2O_4 \cdot C_2H_2O_4$
11	H	$4\text{-}HOC_6H_4CH_2CH_2$	$CH_2CH{=}CH_2$	227-229	7	MeOH	$C_{20}H_{25}NO_3 \cdot 0.5C_2H_2O_4$[g]
12	H	$4\text{-}HOC_6H_4CH_2CH_2$	$2,3\text{-}(CH_2)_3CO$	213-215	41	PrOH	$C_{21}H_{25}NO_4 \cdot 0.5C_2H_2O_4$
13	H	$4\text{-}HOC_6H_4CH_2CH_2$	CH_3CONH	182-184	23	MeOH	$C_{19}H_{25}N_2O_4 \cdot C_2H_2O_4$

[a] Yields based on epoxide. [b] All compounds were analyzed for C, H, N; analytical results were within ±0.4% of the theoretical values. [c] Reported in the literature as HCl.[17,18] [d] Oxalate. [e] Reported in the literature as a free base.[9] [f] Reported in the literature as a free base.[4] [g] Hemioxalate.

Table II. Affinities and Cardioselectivity

compd	K_{app}, μM[a] RVM	K_{app}, μM[a] RLM	cardioselect: RLM/RVM
1	0.24	0.37	1.5
2	0.38	1.8	4.7
3	0.7	0.24	0.3
practolol[b]	18	313	17
4	7	224	32
5	18	88	4.9
6	2.2	34	16
7	0.35	27	77
8	3.9	39	10
9	0.32	17	53
10	2.7	14	5
11	0.20	0.27	1.3
12	0.37	0.56	1.5
13	2.1	16	7.6
propranolol[c]	0.011	0.018	1.6
alprenolol[g]	0.0077	0.02	2.6
bunolol[d,f]	0.015	0.017	1.1
pindolol[e]	0.014	0.093	6.6
4-hydroxybenzylpindolol (HYP)[e]	0.012	0.0059	0.5

[a] Determined to a coefficient of variation greater than or equal to 0.5. [b] Gift of Ayerst Laboratories, N.Y. [c] Purchased from Sigma Chemical Co., Mo. [d] Gift of Warner-Lambert Research Institute, N.J. [e] Gift of Sandoz Pharmaceuticals, N.J. [f] Only *l*-bunolol was used. All other compounds were used in the *dl* form. [g] Gift of Astra Pharmaceutical Products, Mass.

in the cardioselectivity was only twofold (practolol vs. **4**). Perhaps the discrepancy is due to the different tissue preparations and assay techniques used in both studies.[2] Replacing the isopropyl in practolol with 4-hydroxyphenethyl (**5**) did not change the affinity to the RVM receptor but decreased the cardioselectivity.

A striking contrast is provided when the 4-hydroxyphenethyl substituent is introduced into the nonselective β-blockers alprenolol (**11**) and bunolol (**12**). The 4-hydroxyphenethyl group had no effect on cardioselectivity (the ratios of affinity being 1.3 and 1.5, respectively) but caused a 25-fold loss in affinity to the RVM receptor.

Crowther et al.[16] reported that the ortho analogue of practolol was practically devoid of β-adrenoceptor blocking activity in their in vivo assay. Our 4-hydroxyphenethyl analogue of *o*-practolol (**13**) had a slightly higher affinity to both RVM and RLM receptors than its para analogue **5**.

In general, the 4 substitution in the 3-(aryloxy) group results in a differential loss of affinity, leading to cardioselectivity. As demonstrated by Basil et al.[5] and Smith et al.,[6,7] the loss of affinity to the adrenoceptors caused by the introduction of acetamido, carbamoyl, or ureido substitutents in the 4 position can be partially recovered by lengthening the alkyl chain on those substituents. They also suggested that an electron-withdrawing substituent in the 2 position (R_3 of I) of the 3-(aryloxy) moiety further potentiated that effect. Since the caproamido group appeared to be the optimal replacement of acetamido, we prepared compounds **6–8** bearing that group and also **9** and **10**, which also have a chlorine in the 2 position. The increase in affinity of **6** vs. practolol, **8** vs. **5** and **7** vs. **4** to the RVM adrenoceptor is significant. The introduction of chlorine in the 2 position of the 3-(aryloxy) group (**9** and **10**) did not cause a significant change in affinity to either receptor.

By comparing **2** vs. **3**, **4** vs. **5**, **7** vs. **8**, and **9** vs. **10**, it is obvious that the 4-hydroxyphenethyl group decreases the cardioselectivity in all these compounds as compared to those bearing the 3,4-dimethoxyphenethyl group. This

consistent decrease in cardioselectivity does not imply a concomitant decrease in affinity to the RVM receptor. In the case of **8** vs. **7** and **10** vs. **9**, the affinity is decreased by more than eightfold. In contrast, with **5** vs. **4** and **3** vs. **2** there is no significant decrease in the affinities.

In conclusion, the affinity for the RVM receptor among the base compounds **1–3** and among practolol and its derivatives (**4** and **5**) do not differ. The introduction of the caproamido group (R_1) leads to a higher affinity for the RVM, as compared to the respective practolol derivative. In this series of β-blockers, 1-[(3,4-dimethoxyphenethyl)amino]-3-(4-caproamidophenoxy]propan-2-ol (**7**) gives the highest cardioselectivity. This is due to the increase in the affinity to RVM without a comparable increase in the affinity to RLM. The observed synergism of the two substituents, 4-(caproamido) and 1-[(3,4-dimethoxyphenethyl)amino], on cardioselectivity may be due to the effects these substituents have on the interaction of the blocker with the β_1- and β_2-adrenoceptors.

Experimental Section

IR spectra were recorded in KBr disks on a Perkin-Elmer spectrophotometer Model 700 and are consistent with the assigned structures. Liquid chromatography was performed on a Waters Associates ALC 202/6000 and Prep 500 chromatographs. Columns used were Waters Associates μ Bondapak C_{18} and C_{18}/Porasil B for ALC 202/6000 and silica gel for Prep 500. Solvent systems used were: water/MeOH (ALC 202/6000) and MeOH/CH_2Cl_2 (prep 500) of various proportions. Melting points were determined on an Electrothermal capillary melting point apparatus and are uncorrected. Elemental analysis were performed by Galbraith Laboratories, Inc. The results obtained are within ±0.4% of the theoretical values. A typical preparation is given.

1-[(4-Hydroxyphenethyl)amino]-3-(2-allylphenoxy)-propan-2-ol (11). A suspension of tyramine hydrochloride (5 g, 28.8 mmol) and sodium bicarbonate (8.4 g, 0.1 mol) in 50 mL of MeOH was refluxed for 30 min and filtered hot. The filtrate was mixed with 1,2-epoxy-3-(2-allylphenoxy)propane[13] (3.9 g, 20 mmol) in 20 mL of MeOH. The mixture was heated under reflux for 24 h and then evaporated to dryness under reduced pressure. The residue was dissolved in $CHCl_3$ and washed repeatedly with water to remove the unreacted tyramine. The $CHCl_3$ phase was dried ($MgSO_4$) and evaporated to dryness under reduced pressure. The oily residue was chromatographed on silica gel in 10% MeOH in CH_2Cl_2, and the desired fraction converted to the oxalate salt using oxalic acid in MeOH. Obtained crystals were recrystallized from MeOH and chromatographed on C_{18}/Porasil B in water/MeOH (60:40): yield 7%; mp 227–29 °C.

Tissue Preparation. Heart microsomal preparations were obtained by the method of Harden.[19] The hearts were removed from freshly killed (by etherization) rats. The ventricular muscle was dissected free of atria, major vessels, and fat, minced with scissors, and then homogenized (Brinkman Polytron PC-U) in 7 volumes of ice-cold 0.25 M sucrose–Tris buffer (10 mM, pH 7.4) containing 1 mM dithiothreitol (DTT) (buffer 1). The homogenate was washed twice with buffer 1 by centrifugation, and the final pellet was suspended in 7 volumes of 1.7 M sucrose–Tris buffer (pH 7.4, 1 mM DTT). The homogenate was overlayed with buffer 1 and centrifuged in an SW 27 rotor at 25 000 rpm for 1.5 h. The microsomal fraction which formed at the interface of the sucrose solutions was removed with a pasteur pipet and suspended in 10 mM Tris buffer (pH 7.4) in normal saline for assay. Four grams of ventricular muscle suspended in 30 mL (final volume) provides 10^{-10} M receptor.

The lungs were removed from freshly killed animals, dissected free of large bronchi, minced with scissors, and homogenized in 4 volumes of buffer 1. The homogenate was centrifuged at 10 000*g* for 20 min, and the supernatant was centrifuged at 100 000*g* for 1 h. The pellet from 6 to 8 g of lung was suspended in 30 mL of Tris-buffered saline for the receptor assay, giving 10^{-9} M receptor.. All heart and lung preparations were used on the day of preparation.

Determination of Apparent Dissociation Constants. The apparent drug dissociation constants (K_{app}) were determined by competition with [1-^{3}H]dihydroalprenolol ([^{3}H]DHA; New England Nuclear, 36 Ci/mmol). A 0.1-mL aliquot of the drug in 50% EtOH/H_2O was added to test tubes at 50-fold, the desired final concentration. The tissue preparation containing [^{3}H]DHA at 6 to 10 nM was added in 0.5-mL aliquots, incubated at 37 °C for 15 min, and stored on ice until the extent of binding was determined. The amount of [^{3}H]DHA bound was determined by filtration on GF/C filters.[20] Aliquots of 0.1 mL were added to 5 mL of ice-cold saline, rapidly filtered, and washed with 9 mL of ice-cold saline (both operations take less than 10 s). Binding not associated with β-adrenoceptors (amount bound in the presence of 10^{-5} M propranolol) was 20–25% for heart preparations and 10% for lung preparations. Results were plotted as percent specifically bound vs. log of added drug concentration.[21] Apparent dissociation constants were then calculated by curve fitting the plots using the equation % specifically bound = $100 \times [(X + K_d)/[X + K_d(1 + I/K_{app})]$, where X is the measured ligand concentration of [^{3}H]DHA and K_d its dissociation constant determined from double-reciprocal plots in separate studies; I is the final concentration of added drug.

Acknowledgment. We thank Ms. B. L. Schubert and D. A. Simms for their technical assistance. This investigation was supported in part by Grants HL 19127 and HL 22522 awarded by the Heart and Lung Institute, DHEW.

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