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Soft Drugs VI. The Application of the Inactive Metabolite Approach for Design of Soft β-Blockers[1, 2]

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Abstract: The "inactive metabolite approach" was used to design β-blockers. The acidic inactive metabolite of metoprolol [4-(2-hydroxy-3-isopropylamino) propoxyphenylacetic acid] was used as the lead compound. Its esters (alkyl and cycloalkyl) were found active *in vivo* while reverting quantitatively to the same inactive metabolite in plasma. The cyclohexyl ester showed the best activity, which was cardioselective, similar to the parent compound metoprolol. Although most esters had a plasma half-life of approximately 1 min, their activity (antagonism of isoproterenol induced increase in heart rate) following intravenous administration lasted 45-90 minutes, and the maximum β-blockade was observed at 45–60 minutes in both rats and dogs.

The large number of β-adrenergic blocking agents are generally subject to facile, oxidative metabolic degradations. Many of these metabolites possess significant β-blocking activity, and some metabolites, e.g. of bufuralol (3), have longer biological half-lives than the parent drug. These pharmacokinetic properties make it difficult to optimize therapy in individual patients. It is therefore desirable to design β-blockers which are metabolized in a simple, predictable and controllable manner in one step to an inactive metabolite, regardless of the conditions of the patient and other drugs used. This would necessitate, however, avoiding oxidative metabolism. The general "soft-drug" design has these above objectives (4), and one of the most promising design concepts suggested, the "inactive metabolite approach" (4, 5), is eminently suited for the present problem, since many of the β-blockers have acidic, inactive metabolites, which can serve as the lead compounds (4). The principles of the "inactive metabolite approach" are: 1. select a known inactive metabolite of a drug; 2. modify the structure of the metabolite to resemble (isosterically and/or isoelectronically) the parent drug (activation process); 3. design the structure of the new derivatives in such a way that it metabolizes preferentially in one step to the starting inactive metabolite, without going through reactive, toxic intermediates (predictable metabolism); 4. control transport and binding properties, as well as the rate of metabolism and pharmacokinetics by chemical manipulations of the activated moiety.

The approach is applied in the present paper to the case of metoprolol, **1**, a selective β_1-adrenoreceptor antagonist. Its metabolism was extensively studied in rat, dog and man (6, 7), including patients with impaired renal function (8). As shown in Scheme 1, there are four metabolites resulting from the oxidation of metoprolol, among which the O-demethylmetoprolol **2** and α-hydroxymetoprolol **3** have selective β_1-blocker activity, but with 5 to 10 times lower potency than **1** (6, 9), while the acids **4** and **5** are inactive. The phenylacetic derivative **5** is the major metabolite found in the urine, and it can be the lead compound for our "inactive metabolite approach". Accordingly, a series of esters of **5** were prepared. They are expected to have β-blocking activities, and one hydrolytic (i.e., esterase) process deactivates the compounds yielding the starting inactive metabolite. The rate of hydrolysis deactivation could possibly be controlled by the structure of the esters. The β-adrenoreceptor antagonist activities of the esters were determined in rats and dogs, while relative esterase catalyzed cleavage rates were measured *in vitro* in human plasma.

Experimental

Chemistry. All melting points are uncorrected and were obtained with an electrothermal capillary melting point apparatus.

n-Propyl 4-(2-hydroxy-3-isopropylamino)propoxyphenylacetate **(11)**

A mixture of 4-hydroxyphenylacetic acid (9.12 g, 0.06 mol), n-propanol (40 ml) and $SOCl_2$ (2 ml, 0.028 mol) was refluxed for 3 hr and evaporated *in vacuo*. The residue was extracted with ethyl acetate (200 ml), washed with 10 % Na_2CO_3, then dried

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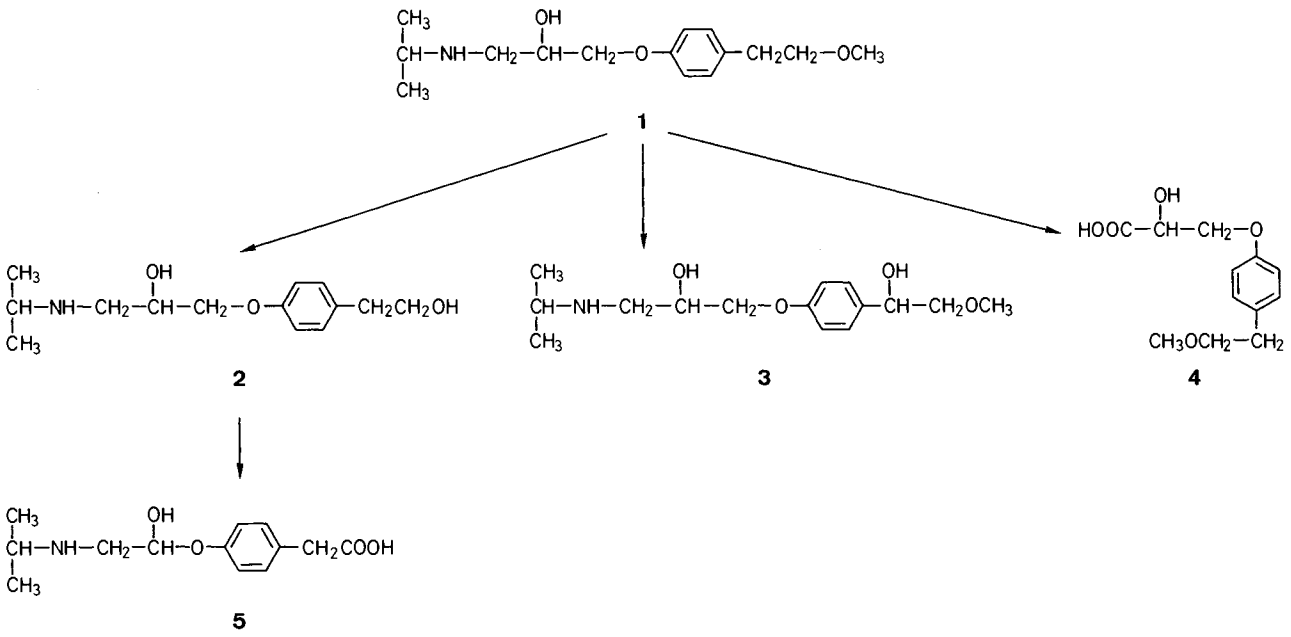


Scheme 1.

with $MgSO_4$, and evaporated *in vacuo* to give n-propyl 4-hydroxyphenylacetate as oil. A solution of n-propyl 4-hydroxyphenylacetate (5.8 g, 0.03 mol) epichlorohydrin (50 ml) was refluxed in the presence of DBU (2 ml, 0.04 mol) for 2 hr. After the excess of epichlorohydrin was removed, the residue was refluxed with i-propylamine (20 ml, 0.23 mol) in n-propanol (50 ml), for 4 hr and evaporated *in vacuo*. The residue was crystallized from n-hexane to yield 3.8 g (41 %) of **11**; mp 61–62 °C.

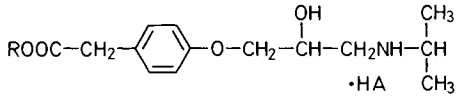
The above method was used with minor modifications for the synthesis of the analogous esters **12–17,** which are all listed in Table I. Some compounds were obtained as oxalic acid salts as described below for **18**.

n-Butyl 4-(2-hydroxy-3-t-butylamino)propoxyphenylacetate **(18)**

The title compound was prepared similarly to **13**, replacing the i-propyl-amine with t-butylamine. The crude **18** was dissolved in Me_2CO_3 and a solution of oxalic acid in Me_2CO_3 was added to it. The crystals were filtered and recrystallized from acetone to yield 9 g (25 %) of **18**; mp 112–114 °C. Anal. $C_{25}H_{41}NO_4 \cdot (COOH)_2 \cdot H_2O$, C, H, N.

Initial Drug Screening Protocol. Thirty-two male Sprague-Dawley rats (Blue Spruce Farms) initially weighing 300–450 g were divided into seven different groups, each for a different drug to be tested: **12** (n=4), **13** (n=4), **14** (n=4), **17** (n=5) and **18** (n=3) (number of animals). The other two groups were controls (n=8, isoproterenol alone pretreated with carrier) and those treated with a known blocker, d,l-propranolol (n=3). Each animal was anesthetized with sodium pentobarbital (45 mg/kg), and the carotid artery was cannulated with PE-50 tubing. This cannula was subcutaneously threaded around the neck and exteriorized dorsally between the shoulder blades. The cannula was filled with a heparin solution (300 μg/ml) and sealed with a solid 22-gauge stylet. The animals were housed in individual stainless steel cages, and two

Table I The synthesized 4-(2-hydroxy-3-isopropylamino) propoxyphenylacetic acid esters.



R	Yield (%)	HA	Anal[a] For	Crystallized From	M.p. °C
5 H[b]	74.0	free base	-		212-213
10 ethyl[c]	43.0	free base	-		44–46
11 n-propyl	41.0	free base	$C_{17}H_{27}NO_4$	n-hexane	61–62
12 i-propyl	30.0	free base	$C_{17}H_{27}NO_4$	petr. ether	59–60
13 n-butyl	42.7	free base	$C_{18}H_{29}NO_4$	chloroformhexane	48–49
14 benzyl	22.0	free base	$C_{21}H_{27}NO_4$	chloroformhexane	73–74
15 cyclohexyl	27.0	oxalate	$C_{20}H_{31}NO_4(COOH)_2 \cdot H_2O$	dimethyl carbonate	131–132
16 2,6-dimethyl-cyclohexyl	22.0	oxalate	$C_{22}H_{35}NO_4(COOH)_2 \cdot H_2O$	acetone	89–91
17 3,3,5,5-tetramethyl-cyclohexyl	53.3	oxalate	$C_{24}H_{39}NO_4(COOH)_2 \cdot H_2O$	acetone	96–97

[a] All compounds gave satisfactory elementary analysis ± 0.4 % C, H, N. NMR and ir spectra consistent with structure.
[b] Described in reference 6.
[c] Described as a synthetic intermediate in A. M. Barrett, J. Carter, R. Haull, D. J. LeCount and C. J. Squire, U. S. Pat. 3,663, 607/May 16, 1972.

days were allowed for recovery from the surgery. Food and water were provided *ad libitum*. On the day of the experiment, the blood pressure and heart rate of each rat were monitored with a pressure transducer (Narco-Bio model P-1000) and the data recorded on a four-channel physiograph (Narco-Bio systems Mark IV). One hour was allowed as an equilibration period before any drugs were administered. All beta blockers were administered intraperitoneally at a dose of 6 mg/kg. Compounds **12** and **14** and d,l-propranolol were dissolved in normal saline, while compounds **13**, **17** and **18** were dissolved in an ethanol : water solution (3 : 1). Depending on the trial, controls were administered the appropriate carrier solution. One hour after administration of the blocker, isoproterenol (Isuprel®, Wintrop Laboratories) was administered subcutaneously at a dose of 25 μg/kg. Blood pressures and heart rate also were recorded at 3, 5, 10, 15, 20, 30, 45 and 60 min after isoproterenol administration. Both control and experimental animals were unrestrained and free moving in their home cage throughout the experiment. Results are shown in Figure 1.

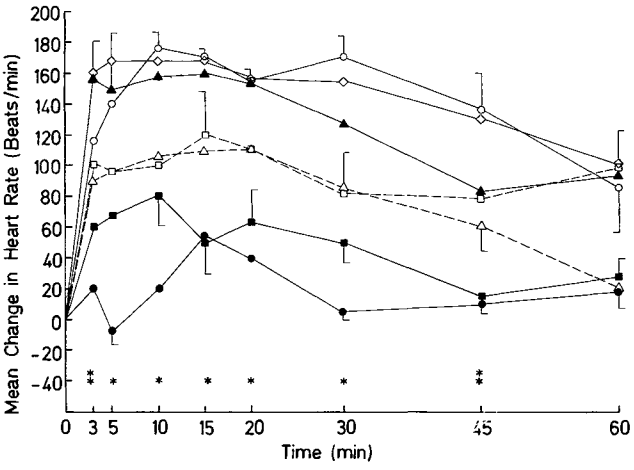


Fig. 1 Mean change in heart rate following administration of isoproterenol (25 μg/kg s.c.). Each group was pretreated with either compound **12** (○); **14** (△); **17** (■); **13** (□); **18** (◇); d,l-propranolol (●) or the control vehicle (▲). Each agent was administered 60 minutes prior to isoproterenol. A oneway analysis of variance (f29, 6) revealed no significant differences in resting heart rates prior to administration of isoproterenol: 345 ± 19; 405 ± 22; 372 ± 19; 340 ± 14; 307 ± 7; 320 ± 23 and 333 ± 17 beat/min, respectively. However, significant differences in the mean heart rate response among the 7 groups were observed: *$p<0.005$; **$p<0.025$. Comparisons between groups were made by the Newman-Keuls procedure with significance set at the 95 % confidence interval. During the first 20 minutes, the group administered compound **17** was significantly different from both control and compound **18** treated groups. The propranolol-treated group was significantly different for the first 30 minutes. Additionally, the group-administered compound **17** and the propranolol-treated group were significantly different than the groups treated with compound **12** and **18** at 10 through 45 minutes following administration of isoproterenol. All data shown are mean ± standard error of the mean.

Duration of Pharmacological Effects of 17. An additional 38 male Sprague Dawley rats (Blue Spruce Farms) initially weighing 268 to 290 gms were anesthetized with sodium pentobarbital (45 mg/kg i.p.), and the carotid artery was cannulated as previously described. On the day of experimentation, after a one hour equilibration period, initial baseline conditions of basal heart rate and mean blood pressures were recorded. The beta blocker (**17**) was administered intraperitoneally at a dose of 6 mg/kg. The drug was dissolved in an ethanol : water solution (3:1). This carrier was also administered in the control rats. Isoproterenol (Isuprel®, Wintrop Laboratory) was then administered (25 μg/kg s.c.) either 15 (n=13), 60 (n=12) or 90 (n=13) min after the beta blocker was administered. Blood pressures and heart rates were recorded at 3, 5, 10, 15, 20, 30, 45 and 60 min after isoproterenol administration in all three trials. Both control and experimental animals were unrestrained and free moving in their home cages throughout the experiment. Heart rates and mean blood pressure were calculated from the data and a one-way ANOVA was determined at each time interval. The results are shown in Figure 2.

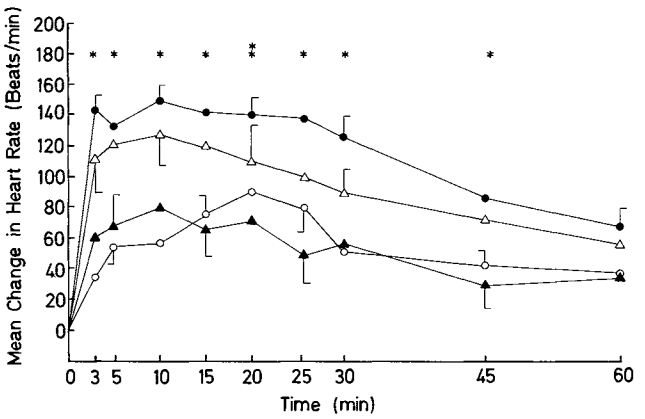


Fig. 2 Mean change in heart rate response in groups pretreated with control vehicle (●); or compound **17**, at 15 (○), 60 (▲) or 90 (△) minutes prior to administration of isoproterenol (25 μg/kg s.i.) at time zero. Resting heart rates were similar in all 4 groups prior to administration of isoproterenol; 342 ± 12; 375 ± 14; 372 ± 19 and 385 ± 17 beats/min, respectively. One-way analysis of variance (f40, 3) revealed a significant difference in response between the 4 groups during the 45 minutes following administration of isoproterenol: *$p<0.005$; **$p<0.025$. Comparison between groups demonstrated an attenuated heart rate response when compound **17** was administered either 15 or 60 minutes prior to the administration of isoproterenol. Data shown are mean ± standard error of the mean.

Kinetic Studies

Analytical Methods. A high pressure liquid chromatography (HPLC) method was developed for the determination of the rate constants. The chromatographic analysis was performed in a system consisting of Waters Associates Model 600-A Solvent Delivery System, Model U-6K Injector and Model 440 Dual Channel Absorbance Detector operated at 254 and 280 nm. A 30 cm × 3.9 mm (internal diameter) reverse phase μBondpak C18 column (Waters Associates), operated at ambient temperature, was used for all separations. When plasma samples were analyzed, the column was protected with a 2.3 cm × 3.9 mm (internal diameter) Guard Column (Waters) packed with μBondapak C18/Corasil packing material. The mobile phase used for the separation of **17** and its degradation product **5** (see Table I) consisted of water, 1-hexanesulfonic acid in acetic acid (B-6 reagent, Waters), 0.1 M triethanolamine and methanol (100 : 1 : 100 : 799). At a flow

rate of 2.0 ml/min **5** and **17** had retention times of 3.95 min and 1.34 min, respectively. For separation of compounds **10–16** and **18** (Table I) and their degradation products, a mobile phase consisting of water, 1-hexanesulfonic acid in acetic acid (B-6 reagent, Waters), 0.1 M triethanolamine and methanol (390:1:10:599) was used. At a flow rate of 2.0 ml/min, **12** had a retention time of 3.79 min, **14**, 4.80 min, **10**, 2.77 min, **11**, 3.85 min, **13**, 5.30 min, **5**, 1.48 min and **18**, 8.39 min.

All solvents and reagents used were of UV or analytical reagent grade and were used as obtained. Water was passed through an ion exchange bed and then distilled.

Determination of the Hydrolytic Rate Constants in Aqueous Solutions. A 0.01 M phosphate buffer and 0.01 N sodium hydroxide solution were prepared from freshly distilled deionized water. The ionic strength was maintained at 0.1 M with sodium chloride. The pH of the phosphate buffer was determined at 37.0 °C with a pH meter standardized at this temperature. For determination of the hydrolytic rate constants, a fresh concentrated solution of the ester in methanol was added to the hydrolytic medium previously equilibrated to desired temperature and mixed thoroughly to result in an initial concentration of about 5×10^{-4} mol · liter^{-1}. All reactions were run under pseudo first order conditions. Samples of 25 µl were injected into the column at various time intervals and the pseudo first order rate constants were determined from disappearance of the compound by linear regression of natural logarithm of the peak height versus time plots. The half-life and standard error of the rate constant were calculated for each run. The results in 0.01 N aqueous sodium hydroxide solution at pH 12.0 and 27.3 °C are listed in Table II.

In 0.01 M phosphate buffer at pH 7.4 and 37 °C, the compounds are hydrolyzed very slowly. The half-life of **14** under these conditions was 13 days and that of **11**, 8.7 days.

Table II. The observed pseudo first order hydrolytic rate constants (k), half-lifes (t½) and the initial concentrations (C_0) in 0.01 N sodium hydroxide at pH 12.0, ionic strength 0.10 M (NaCl) and 27.3 ± 0.2 °C.

Compound	k (min^{-1})	t½ (min)	C_0 (mol. liter^{-1})
10	0.117 ± 0.001[a]	5.91	4.5×10^{-4}
11	0.103 ± 0.001	6.73	4.6×10^{-4}
12	$2.07 \pm 0.03 \times 10^{-2}$	33.5	9.8×10^{-4}
13	$9.27 \pm 0.07 \times 10^{-2}$	7.48	5.0×10^{-4}
14	0.208 ± 0.004[a]	3.33	2.2×10^{-4}
15	$4.96 \pm 0.03 \times 10^{-2}$	14.0	1.7×10^{-4}
16	$9.71 \pm 0.27 \times 10^{-4}$	7.14	8.3×10^{-5}
	$1.09 \pm 0.06 \times 10^{-2}$	63.3	2.8×10^{-5}
17	$1.56 \pm 0.04 \times 10^{-2}$	44.4	3.0×10^{-5}
18	$7.19 \pm 0.04 \times 10^{-2}$	9.64	8.1×10^{-4}

[a] Average of three runs ± SEM. The rest of data are average of four runs ± SEM.

Determination of the Enzymatic Hydrolytic Cleavage Rates in Human Plasma. Freshly collected plasma used was obtained from the Civitan Regional Blood Center, Inc. (Gainesville, Florida) and contained about 80 % plasma diluted with anticoagulant citrate phosphate dextrose solution, U.S.P. The plasma was stored in a refrigerator and used within one week from the date it was collected. During the experiment the hydrolytic activity of the plasma was tested by determining its effect on the hydrolytic cleavage rates of **17** and was found to be constant.

A 50 µl volume of a freshly prepared solution of the compound in methanol was added to 10 ml plasma, previously equilibrated at 37.0 °C in a water bath, and mixed thoroughly to result in an initial concentration of 1×10^{-3} mol · liter^{-1}. One ml samples of plasma were withdrawn from the test medium, mixed immediately with 4.0 ml ice cold 95 % v/v ethanol, centrifuged and the supernatant analyzed by HPLC. The first order hydrolytic rate constant was determined as described above, and the results are listed in Table III.

Table III. The observed first order hydrolytic rate constants (k), half-lives (t½) and the initial concentrations (C_0) in human plasma at 37.0 ± 0.1 °C. The hydrolytic rate constants were obtained by following the disappearance of the compounds by HPLC as a function of time.

Compound	k (min^{-1})	t½ (min)	C_0 (mol-liter^{-1})
10	0.238 ± 0.010[a]	2.91	1.6×10^{-3}
11	0.143 ± 0.005	4,86	2.5×10^{-3}
12	$0.414 \pm 0.001 \times 10^{-2}$	1.67×10^{2}	1.1×10^{-3}
13	0.612 ± 0.016	1.13	1.4×10^{-3}
14	0.236 ± 0.007[a]	2.93	1.4×10^{-3}
15	1.46 ± 0.15[b]	0.47	5.8×10^{-4}
16	—[c]	—	5.9×10^{-4}
	$1.64 \pm 0.14 \times 10^{-2}$	42.2	2.0×10^{-4}
17	0.566 ± 0.027	1.22	9.3×10^{-4}
18	0.351 ± 0.019	1.98	1.5×10^{-3}

[a] Average of three runs ± SEM.
[b] Average of four runs ± SEM.
[c] Essentially no change in the peak height over a period of 3 hours.

Cardiovascular Experiments

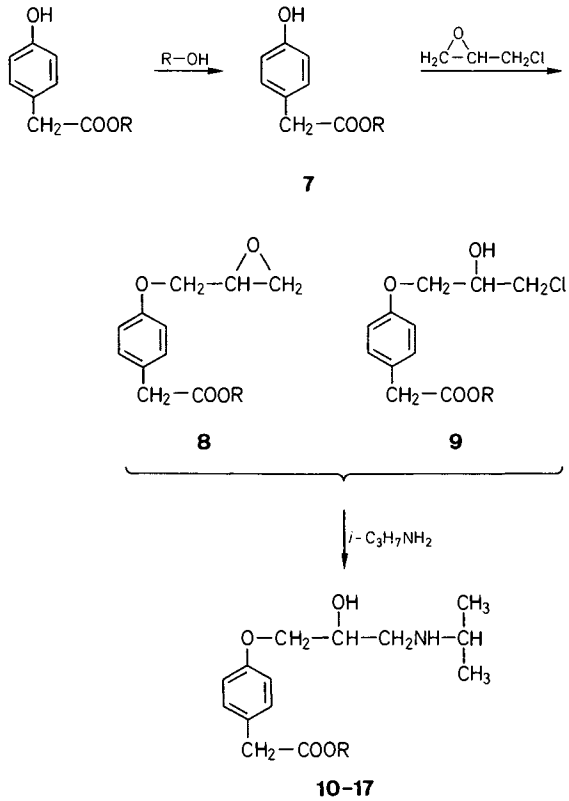
Method. Healthy mongrel dogs were anesthetized with a combination of morphine sulfate (2.0 mg/kg, subcutaneously) followed in 20 minutes with pentabarbital sodium (15 mg/kg, intravenously). Supplemental pentabarbital was given as necessary. A femoral vein was catheterized with polyethylene tubing to the level of the heart for injecting drug solutions. A polyethylene tube filled with heparinized saline was inserted through the femoral artery and advanced to the thoracic aorta for the measurement of arterial pressure. The left carotid artery was cannulated with a Millar® transducer-tip catheter for measurement of left ventricular pressure (LVP). The rising slope of the LVP signal was differentiated to give dP/dt, an estimate of myocardial contractility. Heart rate was determined via a Grass cardiotachometer triggered by the R wave of the lead II EKG. All variables were recorded on a Grass polygraph.

Dose-response curves were constructed for each experimental variable to graded i.v. doses of the β-adrenergic agonist isoproterenol. A period of 3–5 minutes was allowed after each injection for variables to return to baseline. Following this, one of the four experimental β-adrenergic blocking agents was given i.v. over a one-minute period. Abbreviated dose-response curves (2–3 dose points) were again constructed to isoproterenol at 15, 30, 45, 60, 75 and 90 minutes after the administration of the test blockers. Using a 50 beat per minute (bpm) increase in heart rate as criteria, the degree of blockade

was determined by dividing the dose of isoproterenol needed to produce a 50 bpm increase at various times following administration of an antagonist by the dose needed for this effect during the control period. The doses of the experimental β-adrenergic antagonists given were determined from previous experiments to produce an approximate two- or four-fold blockade of the heart rate response to isoproterenol.

Results and Discussions

The compounds of interest were synthesized from 4-hydroxyphenylacetic acid **6**, as shown in Scheme 2. Esterification with the appropriate alcohol of **6** resulted in **7**, which gave a mixture of the intermediates **8** and **9** upon reaction with epichlorohydrin. The reaction of the mixture **8** and **9** with i-propylamine resulted in the ester analogs **10–17** of metoprolol in overall yields shown in Table I.



Scheme 2.

One additional compound, **18**, the t-butylamino analog of **13**, was also prepared and isolated as the oxalate salt.

The hydrolytic stabilities of the esters **10–17** were then determined in aqueous solutions at pH 12.0 and in fresh human plasma at 37 °C. The hydroxide ion catalyzed hydrolysis of the various esters does not show any unexpected results; the rates are generally controlled by the relative steric hindrance at the ester portion. The one interesting case is **16** which consists of a 1 : 2 mixture of two isomers, separable by HPLC. In agreement with this, **16** showed biphasic kinetics. While the starting alcohol is a mixture of isomers potentially six isomers, the ester **16** contains only two, namely the cis-, most likely diequatorial, and the trans-, axial-equatorial, dimethyl cyclohexanol derivatives. The latter possibly is a d,l mixture, inseparable by simple chromatography. It is assumed that the bulky ester group in between the two methyl functions is in equatorial position. The kinetic data are shown in Table II.

The relative hydrolysis rates in human plasma show, however, some unexpected trends (Table III): The i-propyl ester **12** is hydrolyzed up to 100 times more slowly than most of the other esters. Not surprisingly, the isomer of **16**, which is more stable in basic conditions, did not hydrolyze in the plasma within 3 hours.

As expected, the presence of the t-butyl group on the amine did not affect the hydrolysis rates (**13** vs. **18**). It is evident, however, that except for the i-propyl derivative **12**, the rest of the esters hydrolyze very fast in the plasma. Because of high esterase activity in the liver and other organs, esters are generally hydrolyzed faster in the whole body than *in vitro* in plasma. The acid **5** is inactive, and therefore, much β-antagonist activity was not expected, unless some of the esters have an unusually high receptor affinity. Five compounds, **12, 13, 14, 17**, and **18**, were selected for *in vivo* studies in rats and compared to propranolol, a well accepted standard for β-blockers. In the first set of experiments, the compounds were administered intraperitoneally at 6 mg/kg dose, and blood pressure and heart rate were monitored. One hour after administration of the blocker, the agonist isoproterenol was given, and the changes in heart rate and blood pressure were recorded continuously. The main purpose of these experiments was to determine if the compounds show any activity at all at 60 minutes after administration, considering their very short plasma half-lives. The results shown on Figure 1 indicate that the esters **17** and **13**, effectively control the heart rate, although their *in vitro* plasma half-life is in the order of 1 minute. The *in vivo* hydrolysis rates cannot be accurately measured, since the drugs were administered i.p., and the *in vitro* half-life is very short. The extent of this activity is of particular interest, and the time dependence was determined in the case of **17**. Following administration of **17**, as before, the isoproterenol was administered at 15, 60 and 90 minutes, and the blood pressure and heart rates were recorded. It appears (Fig. 2) that at 15 and 60 minutes there is significant activity on heart rate which, however, disappears at 90 minutes. Figure 3 indicates minimal effect on the blood pressure.

Additional *in vivo* cardiovascular experiments were performed with dogs, since they are considered good models for these types of experiments. In order to avoid the uncertainty due to the intraperitoneal administration used for rats, the active compounds were administrated i.v. The changes in heart rate and in left ventricular pressure (LVP) were monitored, as the effect of isoproterenol was antagonized. Together with the selected lead compound, the tetramethylcyclohexyl ester **17**, the simpler homologs **15** and **16** were tested and compared to the ethyl ester, **10**. All four compounds showed β_1-antagonist activity, but the cyclohexyl ester **15** was the most potent in blocking the cardiac effects of isoproterenol. The other three agents had a comparable ability to block isoproterenol-induced tachycardia (Fig. 4).

The time course of the β-adrenergic blockade on heart rate differed among the four compounds. While **10** produced an antagonism which dissipated fairly consistently between 45 and 60 minutes after administration, the duration of action of **16** and **17** was much more variable and generally longer. It is interesting to note that just as in rats (Fig. 2, compound **17**), the maximum blockade in dogs was not at the earliest time following administration, but at 45 or 60 minutes (Table IV).

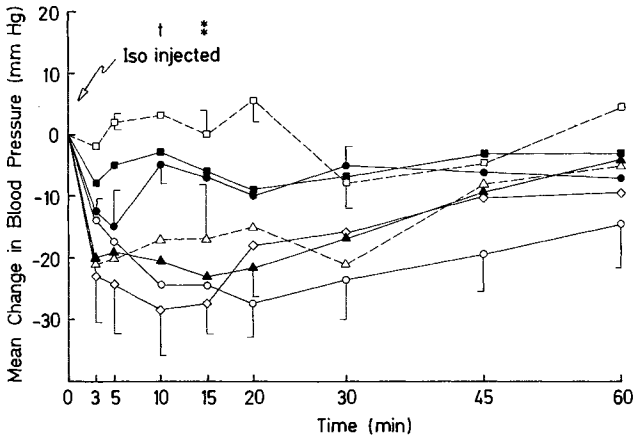


Fig. 3 Mean change in blood pressure response following administration of isoproterenol (25 μg/kg s.c.). Symbols for the groups are the same as those described in Figure 1. Statistical analysis revealed that the groups treated with compounds **14** and **13** were significantly lower ($p<0.15$) than the propranolol and compound **12** treated groups. One-way analysis of variance demonstrated a significant difference between the 7 groups existed at 10 and 15 minutes following administration of isoproterenol: *$p<0.01$; **$p<0.025$. At the 10-minute time interval, groups treated with compounds **17** and **13** were significantly different than the group treated with compound **18**. The group treated with compound **13** was also different from the compound **12**-treated group. At the 15-minute interval, the group pretreated with compound **13** was significantly different from groups pretreated with compound **12, 18** and the control vehicle. Data shown are mean ± standard error of the mean.

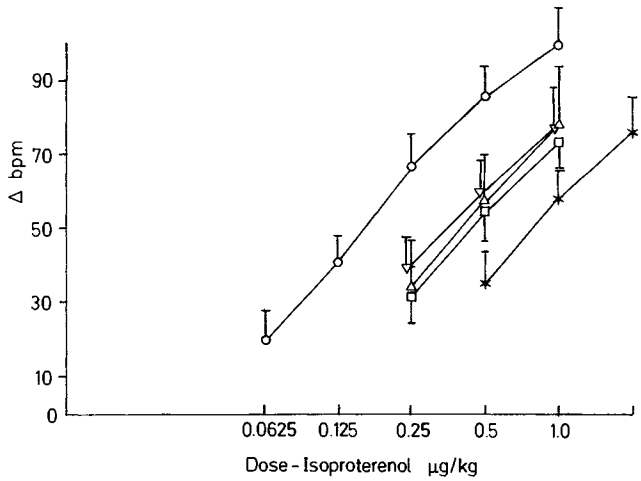


Fig. 4 The β-adrenergic blocking activities of selected "soft" compounds: o—o control; ▽—▽ **17**; △—△ **10**; □—□ **16** and ✳—✳ **15**. Each compound was given to dogs at 1 mg/kg and the increase in heart rate (Δ bpm) by isoproterenol was recorded. Baseline values were 117 ± 6 bpm. Error bars represents S.E. of mean.

Compound **15** had the longest duration of action; approximately 90 minutes were required in most cases for return of heart rate responsiveness to isoproterenol to the control value (Table IV).

All compounds shifted the dose-response curve of isoproterenol on left ventricular contractility (dP/dt) to the right. The extent of this inotropic blockade may be less than the chronotropic (HR) blockade, and the inotropic blockade was more variable. None of the agents had any significant effect on the diastolic depressor response to isoproterenol.

Table IV. Peak antagonist activity[a] and duration of action[b] of selected "soft" β-adrenergic blocking agents.

Compound number	Maximal blockade[a] Range (min)	Mean (min)	Duration[b] Range (min)	Mean (min)
10	(15–15)	15	45–60	~50
15	(15–30)	20	75-90	~85
16	(15–60	38	45–90	~65
17	(30–60)	40	45-90	~70

[a] Time necessary to reach maximal blockade (15 min measurements). Data were obtained from dose-response curves before and after 1 mg/kg of each agent.

[b] Time after each β-adrenergic blocking agent (1 mg/kg) at which response to isoproterenol had returned to control levels.

In conclusion, ester type "soft" blockers designed and synthesized based on an acidic inactive metabolite of metoprolol have been shown to possess significant β-blocking activity. The time of the peak β-blocking activity and the duration of action do not show any correlation with the *in vitro* plasma hydrolysis rates. To the contrary, the longest acting compound, **15**, has the shortest plasma hydrolytic half-life (<1 min). The fast, predictable, hydrolytic deactivation of the circulating active species should result in reduced overall toxicity and adverse drug interactions. In view of the structure of known metabolites **(2–5)** of metoprolol, it is unlikely that oxidative metabolism would compete with the hydrolysis of the soft derivatives **11–18**, but this aspect will be studied when selected compounds will be given orally and i.p. The inactive metabolite approach is currently investigated for other cardiovascular agents.

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References and Notes

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