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IN VITRO PHARMACOLOGIC ACTIVITY OF CONGENER DERIVATIVES AND MODEL CONJUGATES OF PROPRANOLOL AND PRACTOLOL*

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Abstract—Previous studies in our laboratory suggested that synthetized derivatives of isoproterenol and histamine could create agonists more potent and receptor and/or tissue selective than the parent compound. In the present study we have evaluated the hypothesis that our results with isoproterenol and histamine derivatives could be extended to include β-adrenergic antagonists. With this purpose in mind, fourteen derivatives of propranolol and practolol were synthesized and tested in four *in vitro* systems. The congeners and conjugates were tested using biologic assays (blocking of cAMP accumulation) and/or radioligand binding assays in S-49 lymphoma cells and in rat adipocytes, heart and lung which contain β_1 and/or β_2 receptors. Our results indicate that structural modifications distant from the pharmacophore alter the pharmacologic profile of the parent compound. The relative potencies of the derivatives were dependent upon several key factors including the length of the methylene spacer chain and the nature of the substituents on the aromatic ring. The presence of a spacer group with four methylenes resulted in the most active compound in each series when tested on S-49 cells. The derivatives with a paramethyl toluidide group were more potent than the derivatives with a trifluoromethyl toluidide group. The dipeptide derivatives were more potent on adipocyte than S-49 cells, suggesting a preference for β_1 receptors. Some of the same modifications that led to altered potency and which resulted in an increased receptor and/or tissue selectivity using the progenitors isoproterenol or histamine did extrapolate to the beta blockers. Our data suggest that alterations in receptor and/or tissue selectivity must be imparted by the carrier moiety of the drug and may be related to the biochemical microenvironment of the receptors.

A major drawback in the therapeutic use of β-adrenergic agonists and antagonists is that they regulate major physiological functions in many different tissues. We have investigated previously whether unusual modifications of β-adrenergic agonists and histamine could modify the action of these compounds on specific tissues or alter their selectivity or affinity for receptors. We have reported [1–4] that, when isoproterenol and histamine are attached covalently to complex but pharmacologically inert carriers, a number of the derivatives not only retain pharmacologic effects of the progenitor drug but also become tissue selective. The characteristics of the carrier (their size, pK, and hydrophobicity) are the important determinants of the derivatives which alter the potencies and affinities.

The synthetic approach used in modifying isoproterenol [1, 2] and histamine [4] has been extended to the β-adrenergic antagonists propranolol and practolol. In this report, we describe their *in vitro* biologic and receptor binding effects. The effects of these blockers show some parallels with the analogous series of isoproterenol and histamine. We have constructed tissue selective β-adrenergic antagonists, without modifying the aromatic groups of the parent molecules, classically considered the receptor specific portion of the molecule.

MATERIALS AND METHODS

Synthesis of congeners and model derivatives. The propranolol and practolol congeners and congener derivatives were synthesized from the "nor" drug and the appropriate methyl ketone via amination. The synthesis of the ketoamides has been described previously [5, 6]. Norpropranolol was synthesized by the method of Ing and Ormerod [7]. Norpractolol was synthesized from 2-(*p*-nitrophenoxy)-1-phthalimidopropan-2-ol as described by Petrow and Stephenson [8]. In both cases, the products were racemic mixtures and were tested as such. Reductive aminations were performed in the presence of sodium cyanoborohydride (Aldrich) in methanol at 50°.

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Final compounds were purified by either HPLC, flash chromatography, or cystallization of the residue after workup. Propranolol derivatives isolated as the free base (10 and 12) were converted to the hydrochloride salt prior to biologic testing. Compounds were subjected routinely to analytical HPLC for confirmation of purity. Only samples which were >99% pure were accepted for biological evaluation. Final products were characterized by 360 MHz ^{1}H NMR and by elemental analysis where sufficient quantity of material was available. The details of the synthesis of each compound have been submitted to the National Auxillary Publication Service (West Hempstad, NY).

S-49 mouse lymphoma cell assay. S-49 mouse lymphoma cells were centrifuged and resuspended at a density of 2.25×10^6/ml in Dulbecco's modified Eagle's medium (13.3 mg/l) and 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.4) (DMEH) plus 0.1% bovine serum albumin [9]. They were next incubated at 37° for 10 min without drug and were then added to tubes with or without test compounds (with various concentrations of agonists and antagonists) for an additional 6 min and cAMP was measured by a protein binding assay [1]. Isoproterenol (8×10^{-8} M) was added at a concentration equivalent to its ED_{90} of intracellular accumulation of cAMP. Various antagonist derivatives were administered at concentrations ranging from 10^{-14} to 10^{-4} M. Alternatively, some of the congeners were tested by assessing the effects of the antagonist (several different concentrations) on full dose–response curves of isoproterenol (10^{-4} to 10^{-9} M). Representative data are demonstrated in Fig. 1. The concentrations of antagonists required to block 50% of the ED_{90} of isoproterenol were then calculated.

Preparation of rat fat cells for radioimmunoassay. Fat pads were taken from the epididymal fat pads of rats and suspended in 0.9% NaCl. Minced fat (1–2 g) was suspended in 3 ml of Krebs buffer containing 10 mg collagenase. The incubation was for 1 hr at 37° in a rotary shaker. After incubation, the fat cells were filtered through a nylon mesh into small plastic beakers; the filter was rinsed first with a small amount of Krebs buffer and then with 0.25 M sucrose, 10 mM HCl and 1 mM EDTA (pH 7.4) buffer. After centrifugation, the bottom layer was separated and fat cells were resuspended in Krebs buffer.

Fat cells were diluted to 1×10^6 cells/ml and incu-

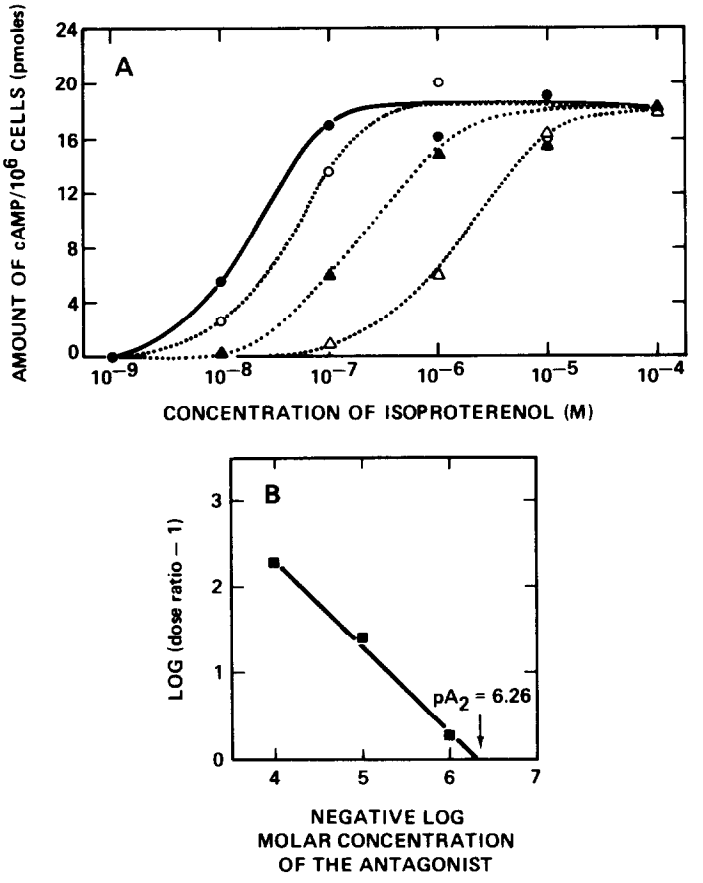


Fig. 1. (A) Dose–response curve for accumulation of intracellular cAMP in S-49 cells in response to isoproterenol (●) and its blockade by a 10^{-5} M (△), 10^{-6} M (▲) and 10^{-7} M (○) concentration of congener 4 (paramethyl toluidide derivative of propranolol) in S-49 lymphoma cells. (B) Antagonist effects of the congener derivative of practolol, congener No. 11, against the cAMP response of S-49 lymphoma cells to isoproterenol. The figure plots log (dose ratio −1) versus molar concentration of the antagonist according to Arunlakshana and Schild [12]. The slope was −0.998 and the pA_2 value 6.26.

bated at 37° for 10 min with 10 mM isobutylmethylxanthine (IBMX) at a final concentration of 20 μM. Incubations with agonist and β-antagonist derivatives were performed as described for the S-49 cells. The intracellular accumulation of cAMP was measured by the radioimmunoassay previously reported [10].

[^{125}I]*Cyanopindolol binding assays.* Binding assays were conducted in membrane fractions from the rat heart, lung, and fat cells and from S-49 lymphoma cells. Membranes were resuspended in buffer containing 50 mM Tris–HCl and 10 mM $MgCl_2$ (pH 7.5). Assays were performed in a total volume of 0.15 ml containing 150 pM [^{125}I]CYP at 37° for 60 min (S-49, fat cells and heart) and 30 min (lung), by which time equilibrium had been achieved (data not shown). Incubations were terminated by washing membrane preparations with 20 ml of buffer over glass fiber filters. Radioactivity on filters was determined in a gamma counter (Micromedic Inc.) [1].

Data analysis. The K_i values of the antagonist were calculated by the Cheng and Prusoff equation [11]. The competitive antagonism was determined by Schild analysis [12].

RESULTS

In vitro *activity of the derivatives of propranolol and practolol on S-49 cells.* The *in vitro* biologic activity of fourteen derivatives was tested in S-49 cells as a model of β_2 receptors. The data are shown in Tables 1 and 2. The *n*-butyl amide (compound 1) and the aromatic amide congener derivatives of propranolol and practolol (compounds 2–7 and 10–13) had a wide spectrum of potencies as β-adrenergic antagonists. The aromatic amide derivatives were synthesized to study the effect of the methylene chain length on the potency. As shown in Tables 1 and 2, the relative potency of *p*-toluidide congener derivatives of propranolol and practolol was dependent upon the length of the methylene chain. The derivatives with four methylene groups adjacent to the carboxyl group (compounds 4, 11 and 13) were the most potent of the series on S-49 cells. The derivatives with three or five methylenes possessed considerably lower activity in the propranolol series and were impotent in the practolol series. Figure 1 shows the blocking abilities of congeners 4 and 11.

The nature of substituents on the aromatic ring was another determinant of the activity of amide congener derivatives in S-49 cells. When the para-methyl substituent (compounds 4 and 11) was replaced by the more electron-withdrawing trifluoromethyl group (compounds 6 and 13), the β-blocking activity decreased. We found no evidence that any of our compounds were partial agonists. Furthermore, a shift of the trifluoromethyl group from the para- (compound 6) to the ortho-position (compound 7) further reduced the potency. Compound 11 was still more potent than practolol as a β_2 inhibitor. The blocking activity of compound 11 was analyzed by the Schild plot [12]. A straight line was observed with a slope of -0.99, and the pA_2 value derived from the X intercept with this regression was 6.26 (Fig. 1B). Consequently, this representative compound appears to antagonize isoproterenol in a competitive fashion. Compounds 8 and 14 with a single amino acid and compound 9 with a dipeptide were less potent than their progenitor drugs.

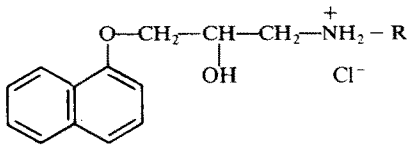
The ability of the derivatives to compete for the binding sites was assessed using [^{125}I]cyanopindolol (ICYP) as a β-ligand to determine if similar relative potencies could be obtained from two different assays of β-antagonism and how they would compare with the K_i values derived from testing the inhibition of cAMP accumulation. If data on cyclic AMP accumulation assays were similar to those from specific receptor binding assays, then the antagonism caused by the new compounds was likely to be caused solely by beta blockade.

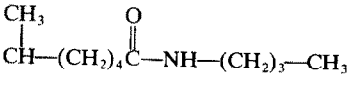
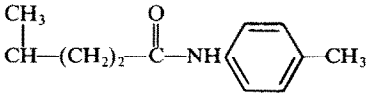
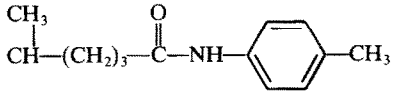
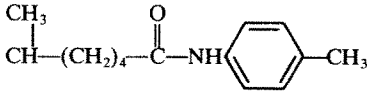
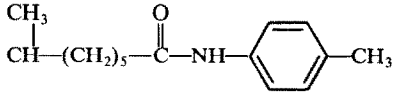
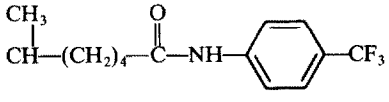
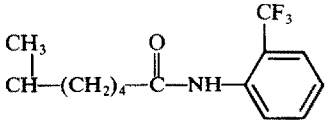
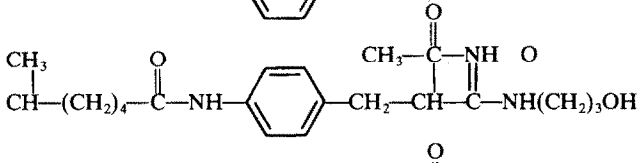
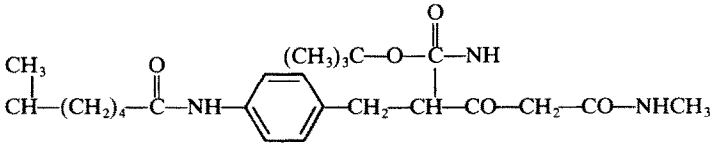
Five propranolol derivatives, one practolol derivative, and the two parent drugs were tested simultaneously in S-49 cAMP assays as well as by radioligand binding assays. As shown in Fig. 2A, there was a good correlation between the relative potencies of the antagonists obtained in the cAMP and radioligand binding assays. We do not know the reasons for the differences in absolute numbers obtained from the cAMP assay versus the radioligand binding in S-49 cells. However, similar variations in results have been reported previously by others [13]. The correlation between the two assays suggests that all the blocking effects of the derivatives were due to classical receptor–antagonist interactions. The alterations in effect specificity and affinities clearly were attributable to the chemical groups attached to the parent drug, even though the ligands themselves had no β-agonist or -antagonist effects (data not shown).

In vitro *activity of the derivatives on the rat fat cells.* Both series of derivatives of β-adrenergic antagonists were tested on rat fat cells to determine their abilities to block isoproterenol-induced intracellular accumulation of cAMP in a model of β_1-receptor antagonism (Table 3). We recognize that β-adrenergic receptors of the rat fat cells may differ from other β_1-adrenergic receptors [14]. These experiments were undertaken to determine whether derivatization of the antagonists could create compounds that were more potent at receptors in fat cells than the progenitor drugs. Compound 8 was the most potent (16×) antagonist of both series on the β_1 receptors of fat cells. This enhanced affinity for the β_1 receptor of the fat cells was much greater than that of practolol or any of its derivatives. In contrast, the *p*-toluidide congener derivative of propranolol with four methylene groups (compound 4) was two orders of magnitude less potent on fat cells than propranolol (Table 3). The congener derivative with a para-trifluoromethyl group (Compound 6) was also a less potent inhibitor of β_1 receptors on fat cells. These derivatives (compounds 4 and 6) seemed to have lost most of the β_1-antagonist action of the progenitor on the fat cells.

The *p*-toluidide congener derivative with four methylenes (compound 11) and the amino acid conjugate of practolol (compound 14) were more potent in fat cells than practolol but they were not as potent in fat cells as compound 8 of the propranolol series (Table 3). In both series (propranolol and practolol derivatives), attaching a single relatively hydrophilic amino acid enhanced the affinity for β_1 receptors.

Table 1. *In vitro* biological activity of congeners and model derivatives of propranolol on S-49 cells*



	Compound-R	K_i	Relative potency
Propranolol		$2.1 \times 10^{-10} \pm 0.28 \times 10^{-10}$	1.0
1.		$1.05 \times 10^{-8} \pm 0.23 \times 10^{-8}$	0.02
2.		$6.51 \times 10^{-4} \pm 0.34 \times 10^{-5}$	3.2×10^{-7}
3.		$6.5 \times 10^{-4} \pm 0.03 \times 10^{-5}$	3.2×10^{-7}
4.		$9.67 \times 10^{-11} \pm 0.12 \times 10^{-11}$	2.2
5.		$1.47 \times 10^{-8} \pm 0.159 \times 10^{-8}$	0.01
6.		$5.04 \times 10^{-9} \pm 0.08 \times 10^{-10}$	0.04
7.		$1.9 \times 10^{-8} \pm 2.1 \times 10^{-8}$	0.01
8.		$2.52 \times 10^{-9} \pm 0.34 \times 10^{-9}$	0.08
9.		$2.4 \times 10^{-9} \pm 0.0024 \times 10^{-9}$	0.09

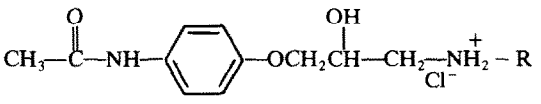
* Biological activity was measured by cyclic AMP accumulation in S-49 cells. Relative potency is expressed as the ratio of K_i for propranolol to K_i for the test compound.

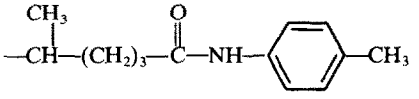
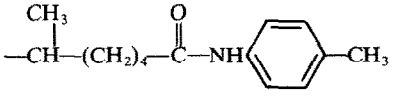
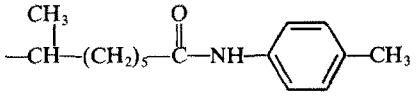
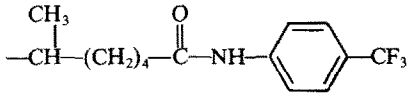
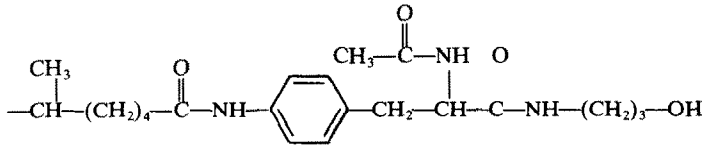
Figure 2B shows that there was a good correlation between the relative potencies of antagonists on fat cells in both cAMP and radioligand binding assays.

Radioligand binding assays in rat lung and heart membranes. None of the derivatives of propranolol was equipotent or more potent than the parent compound in the rat heart or lung membrane binding assays (Table 4). Many of the practolol derivatives were more potent than the parent compound in the heart preparation, and one (compound 13) was more potent in the lung preparation. The paramethyl toluidide with n = 2 (compound 2, Table 1) was ten times more potent on the heart than the lung. When the methylene chain length was increased to n = 3 and n = 4, the selectivity of the effects was lost. All of the congener derivatives of practolol tested (Table 4) were more selective for effects on the heart than the lung. However, compound 13 was 26-fold more potent than practolol in lung preparations.

Comparative pharmacologic activity in various tis-

Table 2. *In vitro* biological activity of congeners and model derivatives of practolol on S-49 cells*



Compound-R	K_i	Relative potency
Practolol	$8.5 \times 10^{-6} \pm 0.163 \times 10^{-7}$	1.0
10. 	Impotent	
11. 	$1.0 \times 10^{-6} \pm 0.11 \times 10^{-7}$	8.5
12. 	Impotent	
13. 	$3.5 \times 10^{-6} \pm 0.22 \times 10^{-6}$	2.42
14. 	$1.4 \times 10^{-5} \pm 0.53 \times 10^{-5}$	0.60

* Biological activity was measured by cAMP assay in S-49 cells. Relative potency is expressed as the ratio of K_i for practolol to K_i for the test compound.

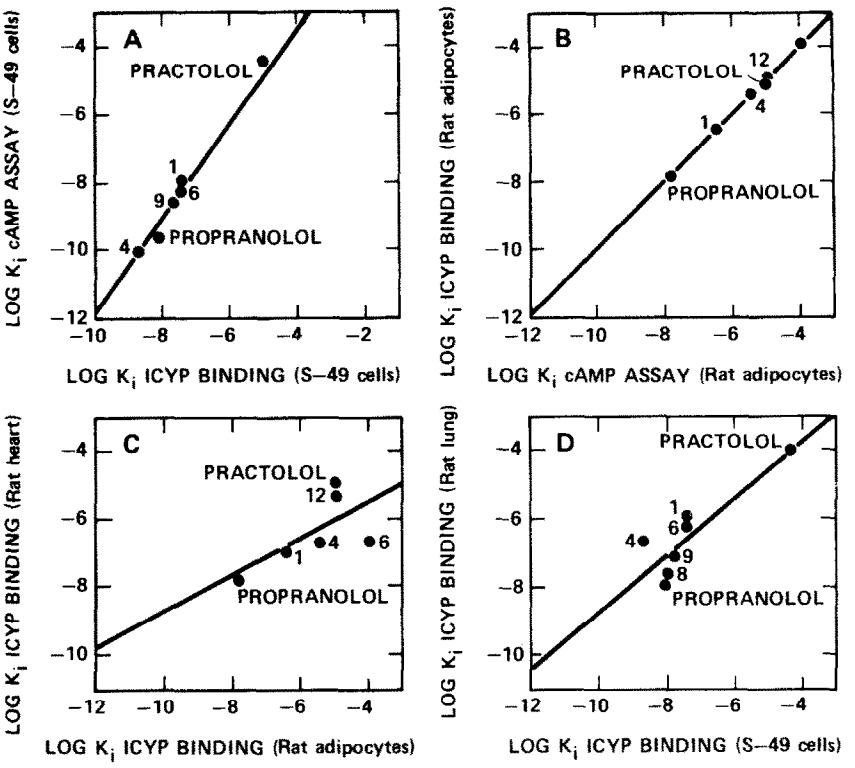
sues. The comparative pharmacology of some of the derivatives in various tissues is summarized in Table 5. Compounds 4 and 6 became much more selective for β_2 receptors on S-49 cells than for β_1 on fat cells. Between tissues with predominant β_1 receptors, the affinities were much higher for heart than for adipocytes. However, as shown in Table 3, compound 8 became sixteen times more potent than progenitor on the rat fat cell β_1 receptors. Compound 8 was less potent than propranolol in heart membranes (Table 4). It will be very important to determine whether these contrasts will hold and can be detected *in vivo*.

As shown in Table 5 and Fig. 2, the activity of practolol was comparable for the β_2 receptors in S-49 and rat lung membrane preparations. Compounds 1, 4 and 6 were more active in S-49 cells than in rat lung, whereas compound 8 and propranolol were more potent in lung than in the S-49 membrane preparation. The derivativization of practolol had enhanced their β_1 specificity in rat heart membrane (Table 4). However, compound 13 indicated a shift of activity from β_1 to β_2 receptors (Table 4).

We also compared β_1-receptor affinity of derivatives of propranolol and practolol in rat fat cells versus rat heart membrane preparations. As shown in Tables 4 and 5 and Fig. 2, while the potencies of propranolol and compounds 1 and 4 were similar for β_1 receptors in rat fat cells and heart preparations, the affinity of a number of the congener derivatives and conjugates was altered. Compound 6 became more potent in rat heart than in adipocyte membranes, whereas compound 8 was more potent on fat cells. The trifluoromethyl anilide derivatives of propranolol [6] were the most selective antagonists for β_1 heart cells receptors over β_1 fat cell receptors.

DISCUSSION

The concept of synthesizing the β-antagonist derivatives was based upon previous reports from our laboratories that some congener derivatives of isoproterenol and histamine were more potent and were receptor or tissue selective [1–4]. The isoproterenol derivatives had unusually high affinities for the receptors they stimulate. The carrier moieties were responsible for the unusual K_d values of the derivatives when compared to their isoprotenerol progenitor [1, 2]. The catecholamine agonist derivatives could be so effect specific as to stimulate only heart or selected lymphoid tissue and not bronchial, adipose, endocrine, or vascular tissues, all of which are stimulated by their progenitor [1, 2]. We considered it possible that the altered pharmacologic characteristics of isoproterenol derivatives could



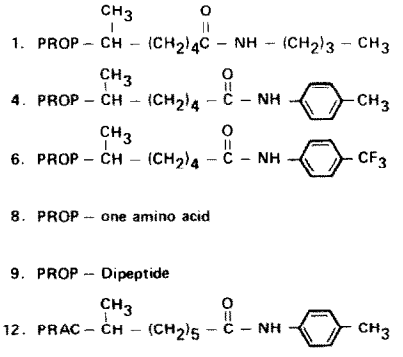


Fig. 2. (A) Log of the K_i of β-adrenergic antagonists and test compounds on ICYP competitive binding experiments versus the log of the K_i on inhibition of cyclic AMP accumulation in S-49 cells ($r = 1.166$). (B) Log of the K_i of β-adrenergic antagonists and test compounds on ICYP competitive binding experiments versus the log of the K_i on inhibition of cyclic AMP accumulation in rat adipocytes ($r = 0.987$). (C) Log of the K_i of β-adrenergic antagonists, congeners derivatives and conjugates on ICYP competitive binding experiments using rat adipocytes versus rat heart membrane preparations ($r = 0.546$). (D) Log of the K_i of β-adrenergic antagonists, congeners derivatives and conjugates on ICYP competitive binding experiments using S-49 lymphoma cells vs rat lung membrane preparations. Both have a predominance of β_2 receptors ($r = 0.837$).

extrapolate to comparable derivatives of the β antagonists since both shared the same receptors for their actions.

We selected a panel of screening tests which would recognize tissue and/or subreceptor (β_1 vs β_2) selectivity of the derivatives. The data showed that major deviations result from the derivatization. In fact, compound 8 lost a significant amount of activity on the S-49 β_2 receptors and became more potent on fat cell β_1 receptors (16×). Of considerable interest were compounds 4 and 6 which were most skewed from their progenitors (Table 5). In order to detect *in vitro* tissue selectivity and specificity, we compared the blocking capabilities of the derivatives in membrane preparations of other tissues responding to catecholamines (heart and lung). The ratios of their affinities in the various tissues are shown in Table 5 and the correlations between different tissues can be observed in Fig. 2, C and D. Although there are marked differences in their tissue and effect specificity *in vitro*, the *in vivo* effects of these compounds have not been tested. If their selectivity is similar to the derivatives of isoproterenol, these compounds could be useful pharmacologic tools and even per-

Table 3. Biological activity of congener derivatives of propranolol β-antagonist and practolol on rat fat cells*

Compound	K_i	Relative potency
Propranolol	$1.7 \times 10^{-8} \pm 2.43 \times 10^{-9}$	1.0
5	$8.5 \times 10^{-7} \pm 1.45 \times 10^{-9}$	0.02
8	$1.07 \times 10^{-9} \pm 4.98 \times 10^{-13}$	15.88
9	$1.74 \times 10^{-7} \pm 4.03 \times 10^{-9}$	0.097
Practolol	$9.33 \times 10^{-6} \pm 3.2 \times 10^{-8}$	1.0
11	$1.90 \times 10^{-6} \pm 0.002 \times 10^{-7}$	4.91
14	$6.92 \times 10^{-6} \pm 9.1 \times 10^{-8}$	1.348

The relative potencies of compounds 1, 4, 6, 10, 12 and 13 were 0.005, 0.0043, 0.0002, 0.11, 0.59 and 0.16 respectively.

* Biological activity was measured by cAMP accumulation in rat fat cells. Relative potency is expressed as the ratio of K_i for the progenitor drug to K_i for the test compound.

Table 4. Inhibition constants of β-adrenergic antagonist derivatives*

	K_i	
Compound	Lung (β_2)†	Heart (β_1)†
Propranolol	$1.24 \pm 0.036 \times 10^{-8}$	$1.60 \pm 0.06 \times 10^{-8}$
1	$1.26 \pm 0.021 \times 10^{-6}$	$1.23 \pm 0.21 \times 10^{-7}$
2	$9.53 \pm 0.321 \times 10^{-7}$	$3.2 \pm 0.064 \times 10^{-8}$
3	$1.12 \pm 0.062 \times 10^{-6}$	$2.13 \pm 0.42 \times 10^{-7}$
4	$3.63 \pm 0.119 \times 10^{-7}$	$2.6 \pm 0.08 \times 10^{-7}$
6	$6.49 \pm 0.064 \times 10^{-7}$	$2.58 \pm 0.60 \times 10^{-7}$
8	$2.4 \pm 0.21 \times 10^{-8}$	$2.13 \pm 0.09 \times 10^{-8}$
9	$8.8 \pm 0.07 \times 10^{-8}$	$5.75 \pm 0.119 \times 10^{-8}$
Practolol	$1.66 \pm 0.59 \times 10^{-4}$	$1.14 \pm 0.03 \times 10^{-5}$
11	$0.88 \pm 0.02 \times 10^{-4}$	$3.41 \pm 0.02 \times 10^{-6}$
12	$2.7 \pm 0.09 \times 10^{-4}$	$4.53 \pm 0.04 \times 10^{-6}$
13	$4.5 \pm 0.42 \times 10^{-5}$	$2.37 \pm 0.16 \times 10^{-6}$
14	$2.0 \pm 0.60 \times 10^{-4}$	$4.76 \pm 0.12 \times 10^{-6}$

* Inhibition constants were measured by a radioligand binding assay using [^{125}I]cyanopindolol as ligand in two rat tissues.

† Rat lung and heart contain 15–20% β_1 and β_2 receptors respectively.

Table 5. Relative potencies of beta-adrenergic antagonist derivatives

	Ratios of inhibition constants (K_i)			
	S-49/Lung	Fat cells/Heart	S-49/Fat cells	Lung/Heart*
Propranolol	0.54	1.0	0.01	0.77
1	0.01	25.0	0.003	10.0
4	0.0054	15.0	0.0005	1.3
6	0.05	414.0	0.0003	2.5
9	0.26	3.0	0.13	1.5
Practolol	0.25	0.81	0.91	14.5
14	0.048	1.45	1.39	42.0

* Rat lung and heart contain 15–20% β_1 and β_2 receptors respectively.

haps selective blockers of the heart or adipose response to catecholamines. It appears that the pharmacologically inert carriers linked to either agonist or antagonist seem to be affecting the beta receptors in a like manner. The agonists and antagonists are not chemically similar. Therefore, these data justify the synthesis and testing of other membrane active agonists that are not chemically related to β-adrenergic agonists or antagonists. As one example, the histamine derivatives with the same carriers as compounds 4, 6, 11 and 13 are tissue selective [4].

Minneman *et al.* [15, 16] have reported that the

calculated ratio of β_1:β_2 adrenergic receptors is almost 5:1 in heart and 1:6 in lung preparations. They have also stressed that predominance of one receptor type does not equate with a given tissue responding only to the agonist of the predominant receptors. While none of the propranolol derivatives were equipotent to the parent compound, several practolol derivatives were more potent than the parent compound in each heart or lung preparation (Table 5). Potency in the catecholamine series closely parallels affinity of the modified agonist for the receptor [1, 2]. With the exception of compounds 1 and 2, most of the propranolol derivatives exhibited a spectrum of activity similar to the parent drug. In the practolol series, all of the derivatives showed higher affinity for heart over lung preparations, and some were more potent than practolol. However, the differences in their activity on β_1 and β_2 receptors in different tissues (Fig. 2) remain unexplained and may suggest a heterogeneity in β-receptor complement.

The differences between the antagonist derivatives and their progenitors parallel our previous observations with catecholamine [1–3] and histamine derivatives [4]. For instance, norepinephrine attached at the amino end of the molecule to a branched alkene chain containing a toluidide terminus has much more inotropic and much less chronotropic effects than norepinephrine [1]. An analogous congener derivative of histamine was active on the H_1 receptors of murine lymphocytes [4] and was inactive on H_2 receptors of lymphocytes and myocardium [4] and on H_1 receptors of rabbit coronary aorta (unpublished observation). Alternatively, aliphatic derivatives of histamine and histamine peptide conjugate retained H_2 activity on the guinea pig myocardium but lost H_2 activity on the murine lymphoid cells. The dipeptide conjugate of the norepinephrine congener derivative became orally active with prolonged *in vivo* cardiac effects when compared to norepinephrine or isoproterenol (manuscript in preparation).

We can conclude from the data that the derivatives of propranolol and practolol are acting on β receptors. As was seen with like derivatives of isoproterenol, the propranolol derivative may, in part, attach to the microenvironment of the β_1 and β_2 receptors [3]. The mechanism for such discrimination by these derivatives is not clear. Hopefully additional data may begin to provide some insight as to what is chemically common to the microenvironment β_1 and β_2 receptors and where modifications can be made that would predictably change the effects on either single type of receptor. Most evidence suggests that even single organs may not have homogenous populations of β_1 or β_2-adrenergic receptors [17, 18], and also that there may be more than two types of β receptors. Ahlquist [19] has even proposed that β-adrenergic receptors in each tissue may have unique pharmacological specificity. Our data seem to confirm Ahlquist's proposal.

Our findings have further strengthened our proposal that, by substituting relatively small functional groups on a parent compound at a point far-removed from what is commonly thought to be the biological recognition site (active portion) of the molecule, new drugs may be made with altered potency, efficacy, or effect and tissue selectivity. Such differentiation could have pharmacologic and/or clinical value.

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