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N-ARALKYL SUBSTITUTION INCREASES THE AFFINITY OF ADRENERGIC DRUGS FOR THE α-ADRENOCEPTOR IN RAT LIVER

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1 The α-adrenoceptor of rat liver plasma membranes was studied by use of the specific α-antagonist [^{3}H]-dihydroergocryptine ([^{3}H]-DHEC). Catecholamines and adrenergic compounds displayed an order of affinity that is typical of an α-receptor. Nevertheless, protokylol, a potent β-adrenoceptor agonist, exhibited a higher affinity than that of adrenaline for α-sites. This result might be due to its bulky substituent on the amino group.

2 Further displacement experiments between [^{3}H]-DHEC and four pairs of drugs differently substituted on the amino group (isoprenaline vs Cc-25, orciprenaline vs fenoterol, AH 3474 vs labetalol, pindolol vs hydroxybenzylpindolol) provided evidence that N-alkyl substitution decreased the affinity for α-sites (20 μM < K_D < 500 μM), whereas an N-aralkyl one increased the affinity (0.17 μM < K_D < 4.6 μM).

3 It is concluded that a substitution on the amino group by a bulky, hydrophobic moiety enhances the affinity of drugs for the α-adrenoceptors.

Introduction

Since the early demonstration by Ahlquist (1948) that agonist catecholamines can be classified into two subgroups, α and β, it has become widely recognized that substitution of the amino nitrogen group of catecholamines by an alkyl group, such as an isopropyl one (*e.g.* isoprenaline) leads to a decrease in the α-agonist activity, with a concomitant increase in β-agonist potency. This has recently been confirmed with the use of [^{3}H]-dihydroergocryptine ([^{3}H]-DHEC), a specific marker for the α-adrenoceptor in the uterus (Williams & Lefkowitz, 1976; Williams, Mullikin & Lefkowitz, 1976), liver (Guellaën, Yatès-Aggerbeck, Vauquelin, Strosberg & Hanoune, 1978), brain (Greenberg & Snyder, 1977) and parotid gland (Strittmatter, Davis & Lefkowitz, 1977). In rat liver plasma membranes, for example, the affinity of isoprenaline for the α-receptor is 50 fold lower than that of adrenaline, while the reverse is found for the β-receptor (Guellaën *et al.*, 1978). However, it appears that the affinity for the α-receptor site does not always vary in a manner opposite to that of the β-site. The present paper, in which we have performed direct competition binding experiments, provides evidence that the introduction of a hydrophobic group, by N-arylation or N-arylalkylation, leads to an increased affinity of the resulting compound for the α-sites. In contrast, a N-alkylation diminishes the affinity of catecholamines and their derivaties for the α-sites.

Methods

Preparation of purified liver plasma membranes

Plasma membranes were prepared from the liver of female Wistar rats (about 100 g body weight) according to the procedure devised by Neville (1968), up to step 11. The purified membrane preparations were suspended in 1 mM $NaHCO_3$ and stored in liquid nitrogen until use.

Binding assay

The binding assay for [^{3}H]-DHEC was carried out as described by Williams *et al.* (1976) with slight modifications (Guellaën *et al.*, 1978). Unless otherwise stated, [^{3}H]-DHEC (5 nM, 12,000 ct/min per 100 μl) were incubated with rat liver plasma membranes (1 mg membrane protein/ml) for 10 min at 30°C in 50 mM Tris–HCl (pH 7.2) containing 10 mM $MgCl_2$ with constant shaking. The final volume of incubation was 0.4 ml. The drugs were dissolved in ice cold water

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and added to the assay just before the experiment. At the end of the incubation, triplicate aliquots (100 µl) were diluted with 4 ml of ice cold buffer and immediately filtered through Whatman GF/C glass fibre filters (25 mm diameter). The filters were immediately washed with 15 ml of incubation buffer at 4°C, dried, placed in scintillation vials, eluted with 1 ml of methanol, and counted in 10 ml ACS aqueous scintillation mixture (Amersham Searle) at an efficiency of 40%.

In the present experiments, specific binding refers to the fraction of bound [^{3}H]-DHEC displaced by 0.1 mM phentolamine which represented 75 to 80% of the radioactivity retained on the filter. In all the results expressed as '[^{3}H]-DHEC bound', only the specific binding was considered. Protein was measured according to Lowry's procedure using bovine serum albumin as standard.

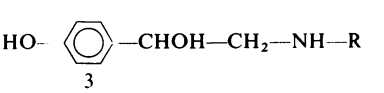
Drugs

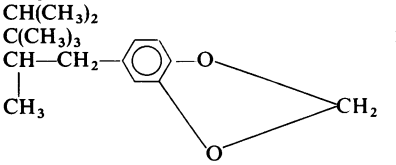
Labetalol (AH 5158A), its parent compound AH 3474 and salbutamol (Allen & Hanburys research Ltd), phentolamine (Ciba-Geigy), protokylol (Lakeside), pindolol, hydroxybenzylpindolol (Sandoz), (±)-isoxsuprine (Mead-Johnson), (±)-nylidrin (U.S.V. pharmaceutical Corps), orciprenaline (Badrial), (±)-Cc-34, (±)-Cc-25 (Phillips-Duphar), fenoterol (Boehringer-Ingelheim), oxyfedrine (Homburg) and mesuprine (Allard) were obtained as gifts; (−)-adrenaline, (−)-noradrenaline, (−)-isoprenaline were from Sigma. All other chemicals were of analytical grade and purchased from Merck (Darmstadt). [^{3}H]-dihydroergocryptine (24 Ci/mmol) was from New-England Nuclear Corp. The purity of this compound was routinely checked by thin-layer chromatography on silica-gel plates, developed in chloroform:benzene:ethanol (4:2:1, v/v/v). In this system, the R_F of [^{3}H]-DHEC was 0.46. Purity was always greater than 95%.

Results

The catecholamine α-adrenoceptor of normal rat liver plasma membranes was identified and characterized by the use of [^{3}H]-DHEC, a potent α-adrenoceptor antagonist (Guellaën *et al.*, 1978). Binding of [^{3}H]-DHEC to the membrane was rapid and saturable. Maximal binding at 30°C and pH 7.2 was obtained with 1,400 fmol bound/mg membrane protein. Scatchard analysis revealed a single class of non-cooperative binding sites with an apparent dissociation constant (K_D) of 4.5 nM. Displacement of [^{3}H]-DHEC from its membrane binding sites allowed an estimation of the K_Ds for various adrenergic agents to be made. As shown in Table 1, agonists competed for the binding sites with the following order of potencies: (−)-adrenaline > (−)-noradrenaline ≫ (−)-isoprenaline; such an order was typical for an α-adrenoceptor. Surprisingly, however, protokylol, a β-adrenoceptor agonist which is 10 fold more potent than isoprenaline on the hepatic β_2-adrenoceptor (Lacombe, René, Guellaën & Hanoune, 1976) exhibited a high affinity for the α-sites: its K_D, 0.45 µM, was three fold lower than that of adrenaline (Table 1). Protokylol differs only from isoprenaline in its bulkier, hydrophobic substituent on the amino nitrogen group.

Table 1 Inhibition of [^{3}H]-dihydroergocryptine binding to rat liver plasma membranes by various α- and β-adrenoceptor agonists



Compounds	3	R	K_D (µM)
(-)-Noradrenaline	OH	H	1.7
(-)-Adrenaline	OH	CH_3	1.5
(-)-Isoprenaline	OH	$CH(CH_3)_2$	74
Salbutamol	CH_2OH	$C(CH_3)_3$	>100
Protokylol	OH		0.45

Incubations were performed as described under Methods in the absence or in the presence of the indicated compounds in various concentrations (ranging from 10 nM to 1 mM). K_D values were calculated according to Cheng & Prusoff (1973) and are the mean of 1 or 2 separate experiments performed in triplicate.

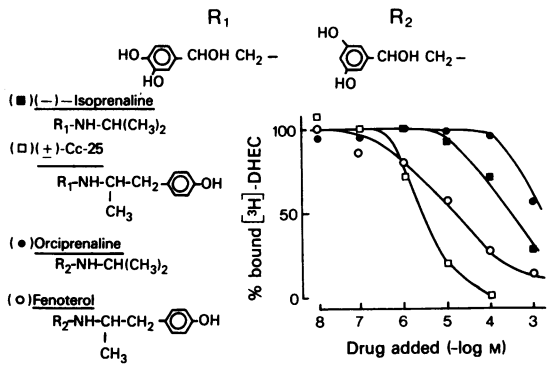


Figure 1 Competition of various β-adrenoceptor agonists for [^{3}H]-dihydroergocryptine ([^{3}H]-DHEC) binding sites in rat liver plasma membranes. Plasma membranes (0.77 mg protein/ml) and 9 nM [^{3}H]-DHEC were incubated with (−)-isoprenaline (■), (±)Cc-25 (□), orciprenaline (●) and fenoterol (○). Results (means of a triplicate determination) are expressed as the percentage of the amount of [^{3}H]-DHEC bound in the absence of drug (1,045 fmol/mg protein).

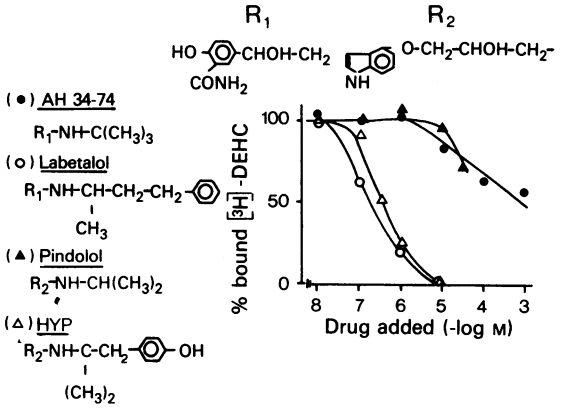


Figure 2 Competition of various β-adrenoceptor antagonists for [^{3}H]-dihydroergocryptine ([^{3}H]-DHEC) binding sites in rat liver plasma membranes. Plasma membranes (1.1 mg protein/ml) and 4.5 nM [^{3}H]-DHEC were incubated with AH 3474 (●), labetalol (○), pindolol (▲) and hydroxybenzylpindolol (HYP) (△). Results (means of a triplicate determination) are expressed as the percentage of the amount of [^{3}H]-DHEC bound in the absence of drug (500 fmol/mg protein).

These findings led us to investigate the possible role of such a substitution in modifying the affinity of adrenergic agents for the α-adrenoceptors in rat liver plasma membranes. For this purpose, we compared the relative affinities of four drugs with those of the same compounds chemically modified by the introduction of a benzyl or hydroxybenzyl group on the amino moiety of the molecule. The parent compounds were a strict β-agonist (isoprenaline), a partial β-agonist (orciprenaline) and two β-antagonists (AH 3474 and pindolol). The modified compounds were Cc-25, fenoterol, labetalol and hydroxybenzyl-pindolol respectively. Competitive binding experiments between these compounds and [^{3}H]-DHEC were performed (Figures 1 and 2) and the dissociation constants, calculated according to Cheng & Prusoff (1973) are depicted in Table 2. It is apparent that, for each pair of drugs, the introduction of an aralkyl substituent on the amino nitrogen group enhanced the affinity of the drug for the α-adrenoceptor by a factor of 50 (e.g. isoprenaline vs Cc-25) to 3000 fold (e.g. AH 3474 vs labetalol).

In order to confirm the importance of this type of substitution for the interaction of drugs with the α-adrenoceptor, we further examined the affinity of several aralkyl substituted compounds endowed with

Table 2 Inhibition of [^{3}H]-dihydroergocryptine binding to rat liver plasma membranes by β-adrenoceptor agonists and antogonists

Compounds	K_D (μM)	*Compounds*	K_D (μM)
(−)-Isoprenaline	74	AH 3474	500
(±)-Cc-25	1.45	Labetalol	0.17
Orciprenaline	500	Pindolol	20
Fenoterol	4.6	Hydroxybenzylpindolol	0.2

The structures of the different compounds are depicted in Figures 1 and 2. Incubations were performed as described under Methods in the absence or in the presence of the indicated compounds in various concentrations (ranging from 10 nM to 1 mM). K_D values were calculated according to Cheng & Prusoff (1973) and are the mean of triplicate determinations.

partial β-agonist properties; the results of these experiments are depicted in Table 3. The drugs tested exhibited a very high affinity for the α-adrenoceptor; two of them, isoxsuprine and nylidrin, had K_D values of 34 and 66 nM respectively, which are only slightly higher than that of phentolamine, a typical α-antagonist (K_D = 9.5 nM) (Aggerbeck, Guellaën & Hanoune, 1978). The K_Ds of the other compounds, Cc-34, oxyfedrine and mesuprine (0.45, 0.8 and 4 μM respectively) were in the range of the values of adrenaline and noradrenaline (Table 1).

Discussion

The results indicate that an aralkyl substitution of the amino nitrogen group of a variety of catecholamine derivatives, agonists and antagonists, leads to an increase in the affinity of the compounds for the α-adrenoceptor sites of isolated liver plasma membranes of the rat. Similar results were obtained by Williams *et al.* (1976), in the case of isoxsuprine, nylidrin, Cc-25 and Cc-34 and by ourselves for labetalol, a known mixed α- and β-antagonist (Aggerbeck *et al.*, 1978). However, a systematic comparison of the various aralkyl-substituted drugs with their parent compounds was not performed in those studies. Our present results, based on competition experiments with membrane α-adrenoceptor binding sites, clearly demonstrate the overall pattern of structure-affinity relationships previously suggested by Ariens (1963, 1967) on the basis of pharmacological experiments, namely that aralkyl substitution of catecholamines could decrease their activity as α-adrenoceptor agonists and, eventually, transform them into competitive antagonists.

The aryl substituted β-adrenoceptor agonist (hydroxybenzylisoproterenol or Cc-34) (Lekfowitz & Williams, 1977) and the antagonist (hydroxybenzylpindolol) (Aurbach, Fedak, Woodard, Palmer, Hauser & Troxler, 1974) have been used to label specifically the β-adrenoceptor sites with high affinity in various tissues. As shown by the present results they may also bind to α-adrenoceptor sites and caution should be exercised when using them in tissue or membrane preparations in which both types of receptors are present.

It is also possible that some partial β-agonists, (e.g. isoxsuprine, nylidrin), may owe part of their pharmacological activities to some α-antagonist properties.

Another extension of the present findings concerns the development of new drugs endowed with α-adrenoceptor antagonist properties together with β-adrenoceptor agonist or antagonist activity. As an example, labetalol, a recently described hypotensive agent (Brittain & Levy, 1976), is the first mixed α- and β-adrenoceptor antagonist. It is quite possible that development of drugs combining α-adrenoceptor blocking and β-adrenoceptor stimulating properties may be beneficial in the treatment of specific diseases such as asthma (Nousiainen, Arnala, Airaksinen & Kokkola, 1977).

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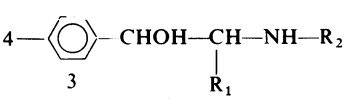
Table 3 Inhibition of [^{3}H]-dihydroergocryptine binding to rat liver plasma membranes by various N-aralkyl substituted β-adrenoceptor agonists



Compounds	3	4	R_1	R_2	K_D (nM)
(±)-Isoxsuprine	H	OH	CH_3	$CH(CH_3)$-CH_2-O-C_6H_5	34
(±)-Nylidrin	H	OH	CH_3	$CH(CH_3)$-$(CH_2)_2$-C_6H_5	66
(±)-Cc-34	OH	OH	H	$C(CH_3)_2$-CH_2-C_6H_4-OH	450
Oxyfedrine	H	H	CH_3	$(CH_2)_2$-CO-C_6H_4-OCH_3	800
Mesuprine	$NHSO_2CH_3$	OH	CH_3	$(CH_2)_2$-C_6H_4-OCH_3	4,000

Incubations were performed as described under Methods in the absence or in the presence of the indicated compounds in various concentrations (ranging from 0.1 nM to 1 mM). K_D values are the mean of triplicate determinations.

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