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Displacement of [^{3}H]dihydroalprenolol binding from rat heart and lung by acebutolol, diacetolol and D-diacetolol

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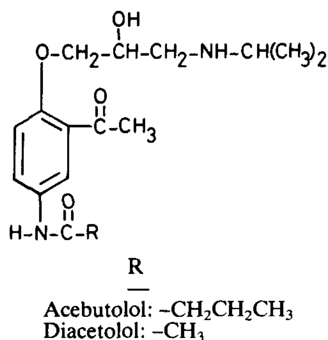
Acebutolol is a cardioselective β-adrenoceptor antagonist with membrane-stabilizing and partial agonist activity (Basil & Jordan 1980). It is effective in the treatment of hypertension, angina pectoris and certain cardiac arrhythmias (Cowling & Leary 1981; DiBianco et al 1982; Mitenko et al 1982; Shapiro et al 1982). Acebutolol is almost exclusively metabolized to diacetolol and the plasma concentration of diacetolol rapidly exceeds that of unchanged acebutolol following oral administration of Sectral (acebutolol HCl, May & Baker) to man (Meffin et al 1978).



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Acebutolol: $-CH_2CH_2CH_3$
Diacetolol: $-CH_3$

The β-antagonistic property and cardioselectivity of acebutolol have been amply demonstrated by its substantially greater potency in blocking the response of the cardiac tissue to β-stimulation than that of the vascular or bronchial tissue (see Basil & Jordan 1980; Cowling & Leary 1981; DiBianco et al 1982). The advantage of cardioselectivity is the lower tendency to precipitate bronchospasm compared with non-selective agents in clinical practice. Diacetolol has a similar cardiac β-adrenoceptor antagonist potency and spectrum of pharmacological activity to that of acebutolol (Basil & Jordan 1980; Mougeot et al 1981; Ohashi et al 1981). Thus, diacetolol may contribute significantly to the pharmacological activity of oral acebutolol in man.

The present study was undertaken to compare the effect of acebutolol, diacetolol, (+)-diacetolol, and several reference β-adrenoceptor antagonists on the specific binding of (−)-[^{3}H]dihydroalprenolol ([^{3}H]DHA) to the rat heart (mainly β_1-adrenoceptors) and lung (mainly β_2-adrenoceptors) preparations. The results of this study further confirm the β_1-adrenoceptor antagonist property and cardioselectivity of acebutolol and diacetolol.

Methods

The binding assay of U'Prichard et al (1978) was generally followed. Test drugs and reagents were prepared in deionized water. The following steps were performed at 0–4 °C unless otherwise stated. The ventricles and lobes of lung from the same male Sprague-Dawley rats were homogenized in 20 ml of ice-cold 50 mM Tris buffer, pH 8·0, with a Brinkman Polytron PT10 (setting 6, 30 s). The homogenates were passed through a fine mesh nylon screen and centrifuged twice at 48 000 *g* for 10 min with an intermediate rehomogenization. The final pellets were resuspended in 100 (heart, 10 mg wet tissue/tube) or 80 (lung, 8 mg wet tissue/tube) volumes of the original wet weight of Tris buffer. To each culture tube were added 100 μl of various concentrations of test drugs or Tris buffer (total binding samples), 100 μl of [^{3}H]DHA (New England Nuclear, NET-507, 43·0 Ci $mmol^{-1}$, final concn 0·5 nM; about 50 000 d min^{-1} tube), and 800 μl of freshly resuspended tissue homogenate. Total binding samples (containing 100 μl Tris buffer) and blank samples (containing 100 μl 5 μM (−)-propranolol) were assayed in triplicate; other samples were assayed in duplicate. The tubes were incubated at room temperature (23 °C) for 20 min, 3 ml of Tris buffer were added to each tube, and filtered rapidly under vacuum through Whatman GF/C filters. The tubes and vacuum filtration wells were each rinsed once with 3 ml of Tris buffer. Filters were then transferred to scintillation counting vials containing 12 ml of Formula-947 (New England Nuclear). The vials were stored in the dark overnight and vortexed. Radioactivity was determined by counting for 10 min in a liquid scintillation counter (Nuclear Chicago Isocap) with counting efficiency of 45–48%. Specific binding of [^{3}H]DHA which was defined as the excess of total binding samples over blank samples containing 5 μM (−)-propranolol was calculated as percent of specific binding of control samples (containing Tris buffer). IC50 values were obtained from at least 3 experiments each containing 4 to 6 drug concentrations. IC50 values were converted to K_i values (inhibition constants) according to the equation as shown in Table 1.

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Table 1. Displacement of (−)-[^{3}H]dihydroalprenolol (0·5 nM) binding from β_1-adrenoceptors in rat heart and β_2-adrenoceptors in rat lung by acebutolol, diacetolol, (+)-diacetolol and reference drugs and their cardioselectivity.

	Displacement of (−)-[^{3}H]dihydroalprenolol binding				
	Heart (mainly β_1)		Lung (mainly β_2)		Cardioselectivity
β-blocking agent	IC50 (nM)[a]	K_i[b]	IC50 (nM)[a]	K_i[b]	(K_i lung/K_i heart)
Acebutol	351 ± 74 (4)	147	3186 ± 192 (4)	844	5·74
Diacetolol	756 ± 109 (4)	317	4750 ± 640 (4)	1260	3·97
(+)-Diacetolol	17 600 ± 5700 (4)	7370	73 200 ± 8800 (4)	19 800	2·69
Butoxamine	3943 ± 739 (3)	1650	2214 ± 205 (3)	587	0·35
Metoprolol	61·0 ± 9·3 (3)	25·6	602 ± 64 (3)	159	6·21
Propranolol	2·81 ± 0·21 (4)	1·18	1·66 ± 0·18 (4)	0·440	0·37
(−)-Propranolol	0·781 ± 0·203 (4)	0·327	0·804 ± 0·111 (3)	0·213	0·65
(+)-Propranolol	128 ± 41 (3)	53·6	47·3 ± 5·2 (3)	12·5	0·23

[a] IC50 values are the concentrations of compounds causing 50% displacement of (−)-[^{3}H]dihydroalprenolol binding. Values given are the means ± standard errors of the number of separate experiments indicated in the parentheses with 4 to 6 concentrations in each experiment.

[b] Inhibition constant $K_i = \dfrac{\text{IC50}}{1 + \dfrac{[(-)\text{-}[^3\text{H}]\text{dihydroalprenolol}]}{K_D}}$

where (−)-[^{3}H]dihydroalprenolol = 0·5 nM and K_D is the equilibrium dissociation constants of (−)-[^{3}H]dihydroalprenolol (0·36 nM in heart and 0·18 nM in lung, from U'Prichard et al 1978).

Results and discussion

The availability of [^{3}H]DHA binding assay (U'Prichard et al 1978) makes it easy to compare the affinity of β-adrenoceptor antagonists to the cardiac (β_1) and lung (β_2) adrenoceptor sites in the same animal at the same time. [^{3}H]DHA was found to bind to β-adrenoceptors in the rat heart and lung preparations. Binding was saturable and equilibrium reached a steady state after 20 min. Binding was linearly related to the wet tissue weight up to 60 mg/tube studied. The K_i values for butoxamine, (+)- and (−)-propranolol (Table 1) agree in general with those obtained by U'Prichard et al (1978). Cardioselectivity was expressed as the ratio between the K_i values in the lung and heart. The higher the ratio, the more cardioselective is the β-blocker.

Metoprolol, a β_1-selective antagonist, was 6·2 times more effective in displacing [^{3}H]DHA binding from the heart than the lung preparations. Butoxamine, a β_2-selective antagonist, was 2·0 times more potent on the lung than the heart. The cardioselectivity of (+)-, (−)-, and (±)-propranolol was less than 1 indicating their non-cardioselectivity. The (+)-isomers (+)-propranolol and (+)-diacetolol) were 15–45 times less active than the racemic mixtures (propranolol and diacetolol), while the (−)-isomer of propranolol was about 3·5 and 2·0 times more active than the racemic mixture in the heart and lung, respectively. These results are all consistent with the literature.

In the heart preparation, diacetolol was about one-half as active as acebutolol and was 2 orders of magnitude less active than propranolol. In the lung, diacetolol was of similar potency to acebutolol and was 3 orders of magnitude less active than propranolol. Although both acebutolol and diacetolol were 2–3 orders of magnitude less active than propranolol, their cardioselectivity is close to that of metoprolol.

The results of this [^{3}H]DHA binding study provide further evidence for the cardioselectivity of acebutolol and diacetolol and are consistent with data previously obtained in animals and man. These results also support the contention that diacetolol may contribute significantly to the pharmacological activity of oral acebutolol in man.

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