Table 1. Inhibition by H 133/22 and H 133/21 of the β_2-adrenoceptor mediated effects of terbutaline on the soleus muscle and on the trachea from guinea-pig in vitro. The mean $pA_2 \pm$ s.e. are shown with the number of experiments in parentheses.

Inhibitor, concn (μmol litre^{-1})	Soleus, pA_2	Trachea, pA_2
H 133/22, 1	7·09 ± 0·07 (5)	6·92 ± 0·25 (5)
H 133/21, 100	5·17 ± 0·13 (5)	5·00 ± 0·18 (5)

The pA_2 values obtained with the soleus were similar to those obtained with the trachea (Table 1) and showed that the β_2-antagonistic activity of H 133/22 is roughly 100 times higher than that of its enantiomorph, H 133/21 ($P < 0.001$). Actually, part of the blocking activity of H 133/21 may be derived from traces of H 133/22. When the estimation of pA_2 is based on a different inhibitor concentration (data from Fig. 1), similar results are obtained indicating a competitive antagonism.

Thus it appears that the antagonistic activity of prenalterol at β_2-adrenoceptors is strictly stereospecific and resides in the same isomer as does the β_1-adrenoceptor agonism. Moreover, both effects appear in the same dose-range, since the pD_2 of prenalterol for the β_1-adrenoceptor agonism on heart tissue is 7·3 (Hedberg et al 1980). In a recent study (Minneman et al 1979), it was shown that H 133/22 binds non-selectively to β_1 and β_2 adrenoceptors. This may explain the complex pattern of agonism and antagonism: H 133/22 has the same affinity for both types of receptors but possesses intrinsic activity at β_1-adrenoceptors only.

The β_2-antagonistic effect of prenalterol may have clinical implications, when it is used as a heart stimulant. In susceptible patients, asthma attacks may be precipitated. However, the degree of β_2-adrenoceptor blockade at therapeutic concentrations of prenalterol remains to be evaluated in clinical trials.

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Prenalterol, a non-selective β-adrenoceptor ligand with absolute β_1-selective partial agonist activity

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In the cat heart, prenalterol has been characterized as a selective β_1-adrenoceptor agonist with about 80% intrinsic activity (Carlsson et al 1977). Results supporting these findings have been reported from pharmacological studies in man (Rönn et al 1979).

To further characterize the interaction of prenalterol with β-adrenoceptors, we have now studied the apparent affinities of prenalterol and isoprenaline for β-adrenoceptors in the cat heart and soleus muscle as derived from receptor binding, their effect on adenylate cyclase activity and their physiological effects.

Binding studies were performed in crude membrane preparations of left ventricular muscle (β_1) and soleus muscle (β_2) (Minneman et al 1979) from reserpinized cats. [^{125}I]Iodohydroxybenzylpindolol (IHYP) was used as the labelled ligand and specific binding was defined as the amount of IHYP displacable by 3×10^{-5} M isoprenaline. Affinity is expressed as pK_d, the negative logarithm of the dissociation constant.

Adenylate cyclase activation was assayed by measuring the conversion of α-[^{32}P]ATP to α-[^{32}P]cAMP in homogenates (10 mg tissue, wet weight ml^{-1} 50 mM Tris-HCl buffer, pH = 7·4) of left ventricular muscle and soleus muscle of the reserpine-pretreated cat (5 mg kg^{-1} i.p. 18 h before death). The effects of prenalterol were studied both in the presence and absence of 3×10^{-5} M isoprenaline. Affinity is expressed either as pK_{act}, which is the negative logarithm of the EC50 for adenylate cyclase stimulation or as pK_i, which is the negative logarithm of the dissociation constant calculated from inhibition of isoprenaline activated adenylate cyclase according to Cheng & Prusoff (1973).

The effects of prenalterol on contractile force were studied in isolated papillary muscles from the right ventricle of reserpinized cats, driven to contract isometrically at 1 Hz by a voltage 20% above threshold. Affinity is expressed as pD_2 (—log EC50).

The apparent affinity values as defined above are given in Table 1.

The displacement of IHYP in the muscle membrane preparations revealed that prenalterol and isoprenaline

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Table 1. Apparent affinities (—log molar concentration; mean ± s.e.m.) for β-adrenoceptors in cat heart and skeletal muscle. n = 6.

	Cat ventricle (β_1)				Cat soleus muscle (β_2)			
	IHYP binding pK_d	Adenylate cyclase pK_{act}	Adenylate cyclase pK_i	Tension development pD_2	IHYP binding pK_d	Adenylate cyclase pK_{act}	Adenylate cyclase pK_i
Isoprenaline	6·47 ± 0·15	6·95 ± 0·07		8·84 ± 0·08	6·30 ± 0·10	6·89 ± 0·05	
Prenalterol	6·59 ± 0·07		6·91 ± 0·04	7·31 ± 0·16	6·52 ± 0·09		6·55 ± 0·10

both show the same degree of specific binding. This indicates that prenalterol binds to the same receptor pool as does isoprenaline in the two tissues. The pK_d-values characteristic of prenalterol affinity for myocardial and skeletal muscle β-adrenoceptors were found to be strikingly similar. This was true also for isoprenaline.

The adenylate cyclase activity was dose-dependently increased by isoprenaline and the pK_{act} values were similar in the two tissues. Our assay failed to detect any statistically significant activation of the myocardial and soleus adenylate cyclase with prenalterol concentrations up to 3×10^{-4} M, but revealed a dose-dependent inhibition of the isoprenaline-activated enzyme. The calculated pK_I-values in the two tissues differed slightly from each other, the reason for which will have to be further evaluated.

In the papillary muscle preparation, prenalterol showed positive inotropic activity with a maximal effect amounting to 82 ± 3% of that of isoprenaline. The pD_2-value of isoprenaline was markedly higher than its pK_d and pK_{act}-values, whereas the prenalterol pD_2-value was only slightly higher than its pK_d and pK_I.

The effect of prenalterol on contractile force in the papillary muscle preparation is in agreement with earlier findings (Carlsson et al 1977) and indicates that prenalterol is a partial β_1-adrenoceptor agonist with high intrinsic activity. Preliminary data suggest that prenalterol, in contrast to isoprenaline, has no effect on subtetanic contractions in isolated, electrically driven (45 V, 12 Hz during 1·5 s every 22 s) strips of the cat soleus muscle. This finding is in agreement with observations in the guinea pig soleus muscle using the racemate of prenalterol, H 80/62 (Waldeck 1977) and supports the observation that prenalterol has no agonistic activity on β_2-adrenoceptors (Carlsson et al 1977).

The cardioselective effects of prenalterol do not seem to be due to a selective affinity for β_1-adrenoceptors as the compound also binds to the β_2-adrenoceptors in the soleus muscle with equal affinity. Our findings suggest that its cardioselectivity is due to the fact that prenalterol has stimulatory activity on β_1- but not on β_2-adrenoceptors.

Kaumann (1978) discussed the possible occurrence of spare β-adrenoceptors in the kitten heart and postulated that a maximal physiological effect with isoprenaline stimulation may be achieved by activation of about 10% of the β-adrenoceptors available in the myocardium. A partial agonist may consequently induce its maximal effect by activating less than 10% of the β-adrenoceptors.

This spare receptor hypothesis may offer an explanation for the lack of significant adenylate cyclase stimulatory activity produced by prenalterol in heart muscle. The hundredfold difference between the EC50-value for the contractile response of isoprenaline and the K_{act} and K_d-values implies that the maximal effect on contractile force is obtained with only a modest increase of cAMP and receptor occupancy (ca 10%). A partial agonist such as prenalterol, with about 80% intrinsic activity, would therefore only be expected to induce a marginal increase in cAMP formation.

The present findings indicate that prenalterol binds non-selectively to both β_1- and β_2-adrenoceptors, but is capable of activating β_1-adrenoceptors only. The available animal and clinical pharmacological data indicate that the β_2-adrenoceptor affinity of prenalterol does not interfere with its practical use as a cardiac stimulant.

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