# On the stereospecificity of the $\beta_2$-adrenoceptor blocking properties of prenalterol

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Prenalterol (H 133/22) is the laevorotatory (−)-isomer of 1-(4-hydroxyphenoxy)-3-isopropyl-amino-2-propanol; its racemic form is known as H 80/62. The compound is a selective $\beta_1$-adrenoceptor agonist (Carlsson et al 1977) with no agonistic effect on $\beta_2$-adrenoceptors (Waldeck 1977; Al-Jeboory & Marshall 1978). However, when we studied the $\beta_2$-adrenoceptor mediated depression of subtetanic contractions of the guinea-pig soleus muscle produced by terbutaline (for review see Bowman 1980), we found that H 80/62 antagonized the effect (Waldeck 1977). In the present study we have compared the $\beta_2$-adrenoceptor blocking property of H 133/22 with that of its (+)-isomer, H 133/21.

The soleus muscle and the trachea from guinea-pigs (male, about 200 g) were prepared for the recording of isometric contractions in vitro. Subtetanic contractions were evoked in the soleus muscle by transmural field-stimulation and tracheal tone was induced by 2·6 $\mu$mol litre$^{-1}$ of pilocarpine (for details see Waldeck 1976; Holmberg et al 1980). Terbutaline was added cumulatively, alone or in the presence of H 133/22 (1 or 10 $\mu$mol litre$^{-1}$) or H 133/21 (10 or 100 $\mu$mol litre$^{-1}$) 15 min before the first dose of terbutaline. The effect on the soleus muscle, depression of subtetanic contractions, was measured and the result was expressed as per cent of the maximum effect elicited by a single dose of 2·3 $\mu$mol litre$^{-1}$ of terbutaline on that muscle. The effect on the trachea was calculated as per cent of the total relaxation achieved after 10 $\mu$mol litre$^{-1}$ of isoprenaline added at the end of the cumulative addition of terbutaline. Dose-response curves were constructed and pEC30 and pEC50 for the soleus and the trachea, respectively, were estimated (cf. Holmberg et al 1980).

H 133/22, 10 $\mu$mol litre$^{-1}$, had no effect on the soleus muscle itself but caused a parallel shift to the right of the dose-response curve for terbutaline ($P < 0.001$, Fig. 1). At the same concentration, 10 $\mu$mol litre$^{-1}$, H 133/21 was much less potent in this respect ($P < 0.001$). These results confirm our previous results with H 80/62 (Waldeck 1977) and indicate that the antagonistic activity resides in the (−)-isomer, H 133/22. The results on the trachea were similar to those on the soleus muscle indicating a preferential antagonistic activity of H 133/22 over H 133/21 ($P < 0.001$, Fig. 1). H 133/22 had no significant relaxing effect on the trachea, which indicates that $\beta_1$-adrenoceptors play little or no role in this preparation.

Al-Jeboory & Marshall (1978) were unable to block the effect of isoprenaline on the guinea-pig isolated soleus with H 133/22, and they suggested that the antagonistic effect of the racemate H 80/62, (Waldeck 1977), resides in the (+)-isomer. However, their preparation appears to have been much less sensitive to isoprenaline than ours, thus requiring a higher concentration of the antagonist.

In other experiments on preparations of the soleus and the trachea, the antagonistic activities of 1 $\mu$mol litre$^{-1}$ of H 133/22 and 100 $\mu$mol litre$^{-1}$ of H 133/21 were estimated. These concentrations were almost equipotent in inhibiting the effect of terbutaline and yielded an agonist dose-ratio (DR) of about 10. From these data, $pA_2$ values were calculated according to the equation $pA_2 = \log (DR-1) - \log [I]$, where [I] represents the molar concentration of the antagonist (cf MacKay 1978). For the calculation of the dose-ratios, the mean control pEC30 and pEC50 values for terbutaline were used viz. 7·35 ± 0·06 s.e. for 7 soleus muscle preparations, and 7·01 ± 0·12 s.e. for 7 trachea preparations.

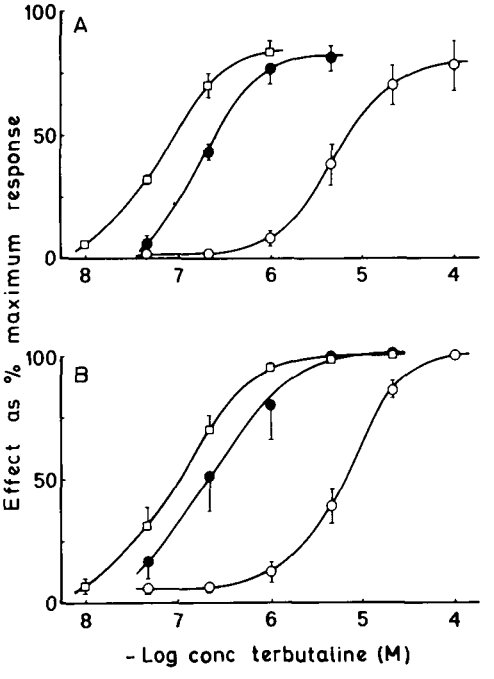


FIG. 1. Inhibition by H 133/22 and H 133/21 of the $\beta_2$-adrenoceptor mediated effect of terbutaline on A, the soleus muscle and B, the trachea from guinea-pigs in vitro. Each point represents the mean ± s.e. from 5–7 experiments. Control (□), 10 $\mu$mol litre$^{-1}$ H 133/22 (○) and, 10 $\mu$mol litre$^{-1}$ H 133/21 (●).

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Table 1. Inhibition by H 133/22 and H 133/21 of the $\beta_2$-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 ($\mu$mol 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 $\beta_2$-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 $\beta_2$-adrenoceptors is strictly stereospecific and resides in the same isomer as does the $\beta_1$-adrenoceptor agonism. Moreover, both effects appear in the same dose-range, since the $pD_2$ of prenalterol for the $\beta_1$-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 $\beta_1$ and $\beta_2$ 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 $\beta_1$-adrenoceptors only.

The $\beta_2$-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 $\beta_2$-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 $\beta_1$-selective partial agonist activity

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In the cat heart, prenalterol has been characterized as a selective $\beta_1$-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 ($\beta_1$) and soleus muscle ($\beta_2$) (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 $3 \times 10^{-5}$ 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 $3 \times 10^{-5}$ 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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