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Pharmacological isolation of a single β-adrenoceptor subtype makes Schild plots which have slopes of 1 possible for selective β-adrenoceptor antagonists in carbachol-contracted guinea-pig trachea

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The effects of selective β-adrenoceptor agonists and antagonists on isometric relaxation of carbachol-contracted, guinea-pig isolated trachea were studied. An attempt was made to pharmacologically isolate the β_1- and β_2-adrenoceptors by use of appropriate selective agonists and antagonists. The slopes of the Schild plots for butoxamine vs noradrenaline and terbutaline as well as for practolol vs terbutaline did not differ significantly from 1, the predicted value for a competitive antagonist acting at a single receptor type. These data differ from a previous report suggesting that slopes of less than 1 are characteristic for selective β-adrenoceptor antagonists in carbachol-contracted guinea-pig trachea. They support the hypothesis that the low slopes in this preparation are due to interaction of the agonist with both β_1- and β_2-adrenoceptors rather than the state of contraction of the trachea.

The pA_x method of Arunlakshana & Schild (1959) provides a widely used measure of antagonist potency. In addition, it predicts that the regression of log (concentration ratio − 1) on log molar concentration of a competitive pharmacologic antagonist should have a slope of 1. However, several laboratories studying guinea-pig trachea have reported Schild plots with slopes of less than 1 for presumably competitive adrenoceptor antagonists (Patil 1968; Buckner & Patil 1971; Imbs et al 1977; O'Donnell & Wanstall 1980). Different explanations for this discrepancy have been suggested.

Furchgott (1970) enumerated six experimental criteria necessary for obtaining meaningful results from such studies, including assurance that the response to the agonist is due solely to its direct action at one type of receptor. Furchgott (1976) also published evidence suggesting that guinea-pig trachea possesses both β_1- and β_2-adrenoceptors and that the ratio varies from animal to animal. He suggested that the Schild plot for a selective antagonist obtained versus a non-selective agonist in such a tissue would have slope of less than 1 because interaction of the agonist with both receptors would obscure the competitive interaction of agonist and antagonist at the receptor-of-interest.

Alternatively, O'Donnell & Wanstall (1980) reported interesting differences between Schild plots obtained for selective β-adrenoceptor antagonists in carbachol-contracted and intrinsic-tone guinea-pig trachea. Their findings suggest that Schild plots with slopes of less than 1 are characteristic of selective antagonists when obtained in carbachol-contracted trachea.

I am unaware of any study examining this question in guinea-pig trachea that fully satisfies the criteria of Furchgott, including blockade of all receptors which might influence the observed response. While most of the studies cited above included an antagonist to block any α-adrenoceptors present, they did not include an antagonist to block the complementary β-adrenoceptor subtype. The study described in this report was designed to satisfy this criterion. Thus, the Schild plot for the β_2-selective antagonist, butoxamine, obtained versus the β_2-selective agonist, terbutaline, in the presence of an effective concentration of the β_1-selective antagonist, practolol, is reported. Similarly, practolol is examined versus the β_1-selective agonist, noradrenaline, with the β_2 receptors blocked by butoxamine. Data are presented which differ in some respects from those reported by O'Donnell & Wanstall (1980).

MATERIALS AND METHODS

Tracheae were removed from male guinea-pigs (Hartley, 400–600 g), cleaned, halved and mounted on clips in tissue baths containing modified Krebs solution bubbled with 5% CO_2 in O_2 and maintained at 37 °C and pH 7·4. The animals were treated with reserpine (1 mg kg^{-1} I.P.) 24 h before the experiment. The bathing solution contained the following (mM): NaCl 118, KCl 4·7, $MgSO_4$ 1·2, KH_2PO_4 1·2,

$CaCl_2$ 2·5, $NaHCO_3$ 25, glucose 11, EDTA 0·02, phentolamine 0·003—to block α-adrenoceptors, cocaine 0·01—to block neuronal uptake, and metanephrine 0·03—to block extraneuronal uptake. In addition, in some of the experiments the bathing solution also contained either (±)-practolol (5×10^{-6} M) or (±)-butoxamine (4×10^{-6} M), depending upon the β-adrenergic agonist used. Preliminary experiments indicated that these concentrations were the pA_{12} and pA_7, respectively, obtained vs the agonist selective for the same β-adrenoceptor subtype.

The tissues were allowed to equilibrate for 2 h at 4–6 g of tension after being placed in the baths. Cumulative concentration-response curves (CCRC) were obtained by first contracting the tracheae with carbachol ($2{\cdot}5 \times 10^{-7}$ M) and then adding the β-adrenoceptor agonist to the baths in a cumulative fashion (van Rossum 1963).

Change in isometric tension was measured with a Narco Model F-60 myograph and recorded on a Narco Physiograph. Response was calculated as the relaxation (i.e. the decrease in grams of tension from the carbachol baseline) caused by each concentration of agonist expressed as a percent of the relaxation caused by a maximally effective concentration of aminophylline given at the end of each run.

The second of two initial CCRC's was used as the control for determining the concentration ratio caused by each subsequent concentration of antagonist. When antagonists were studied, the tracheae were allowed to equilibrate in the presence of each concentration for 1 h. Analysis of variance of data from preliminary studies following this same experimental protocol, but without addition of the antagonist, indicated that any change in sensitivity to the agonist with time and exposure was negligible.

The drugs used and their sources were: (−)-noradrenaline-HCl ((−)-arterenol; Sigma), (±)-practolol (gift of ICI), (±)-butoxamine-HCl (gift of Burroughs Wellcome), (±)-terbutaline sulphate (Brethine; gift of Geigy Pharmaceuticals), phentolamine HCl (Regitine; gift of CIBA Pharmaceutical), cocaine-HCl, (±)-metanephrine-HCl (Sigma), EDTA (disodium salt, dihydrate; Sigma), carbamylcholine chloride (carbachol; Sigma), aminophylline ($(theophylline)_2$–ethylenediamine; Sigma). Glucose, NaCl, KCl, $MgSO_4$, KH_2PO_4 and $NaHCO_3$ were obtained from Mallinckrodt.

All agonist and antagonist solutions were made fresh from the crystalline powder on the day of the experiment using Krebs solution and were kept on ice during the experiment.

A least squares linear regression analysis (Goldstein 1967) was used to calculate the line of best fit, its slope and standard error using the combined data from a number of animals and experiments. pA_2 values and their 95% confidence limits were calculated as described by MacKay (1978).

RESULTS

Fig. 1 shows the CCRC's for the selective β-adrenoceptor agonists, terbutaline and noradrenaline, after blockade of the complementary β-adrenoceptor subtype. The negative log ED50 values for these two agonists before and after blockade are given in Table 1. Blockade of the complementary β-adrenoceptor subtype had no statistically significant effect on the potency of the two agonists.

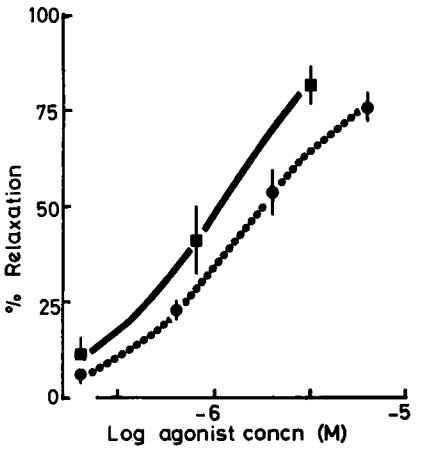

FIG. 1. Cumulative-concentration response curves for noradrenaline and terbutaline in carbachol-contracted guinea pig isolated trachea after blockade of the complementary β-adrenoceptor subtype. Guinea-pig isolated tracheal preparations were contracted with carbachol; when the response had stabilized, the appropriate adrenoceptor agonist was added in a cumulative manner. ■——■, (−)-noradrenaline in the presence of butoxamine (4×10^{-6} M); ● . . . . ●, (±)-terbutaline in the presence of practolol (5×10^{-6} M). Cocaine and metanephrine were present to block neuronal and extraneuronal uptake, respectively. Phentolamine was present to block α-adrenoceptors. Percent relaxation is the decrease in grams of tension from the carbachol baseline caused by each concentration of agonist expressed as percent of the decrease in tension caused by a maximally effective concentration of aminophylline. Mean values of 7 and 8 experiments are represented by the points; standard errors of the mean are represented by the vertical bars.

Schild plots for butoxamine and practolol obtained vs noradrenaline and terbutaline are shown in Fig. 2. The data obtained from these regressions are summarized in Table 2. Individual pA_2 values were calculated from each point on the respective Schild plot, and the regression of these values on the log of

Table 1. Potencies of (−)-noradrenaline and (±)-terbutaline in carbachol-contracted guinea-pig trachea.

Agonist	Negative Log ED50 (95% Conf. Lim.)[a] − β-Blocker[b]	+ β-Blocker[b]
(−)-Noradrenaline	6·27 (6·58, 6·12)	6·00 (6.14, 5·85)
(±)-Terbutaline	5·83 (5·92, 5·74)	5·73 (5·82, 5·64)

[a] Values calculated from data shown in Fig. 1 as the regression of percent relaxation on log dose over the linear portion of the CCRC (Goldstein 1967).
[b] Indicates the presence (+) or absence (−) of the complementary β-adrenoceptor blocker.

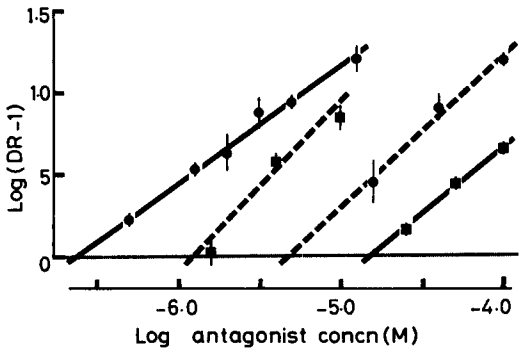

FIG. 2. Schild plots for practolol and butoxamine using noradrenaline and terbutaline as agonists in carbachol-contracted guinea-pig isolated trachea. Shown are the lines of best fit calculated using a least squares regression analysis of combined data obtained in a number of animals and experiments. The points and vertical lines represent the means and standard errors of the values for log (DR − 1) obtained for the respective antagonist molar concentration. ●——●, (±)-practolol vs noradrenaline in the presence of 4×10^{-6} M butoxamine (28 data points, 3 to 11 tissues); ■ - - - ■, (±)-butoxamine vs terbutaline in the presence of 5×10^{-6} M practolol (20 data points, 4 to 12 tissues); ● - - - ●, (±)-butoxamine vs noradrenaline (12 data points; 4 tissues); ■——■, (±)-practolol vs terbutaline (12 data points, 4 tissues). Guinea-pig isolated tracheal preparations were contracted with carbachol; when the response had stabilized, cumulative concentration-response curves were obtained for the appropriate adrenoceptor agonist before and after addition of the adrenoceptor antagonist. The tissues were allowed to equilibrate in the presence of the antagonist for one hour before obtaining the concentration-response curve. Cocaine, metanephrine, and phentolamine were present as described in Fig. 1.

the appropriate antagonist concentration (log [I]) was then determined (MacKay 1978). There was no significant dependence of pA_2 on log [I] except in the case of practolol vs noradrenaline in the presence of butoxamine. Consequently, the best estimates of the pA_2 for all but this combination were taken as the mean values with 95% confidence limits. The dependence of pA_2 on log [I] for practolol vs noradrenaline precludes using the mean pA_2 to characterize quantitatively the interaction of practolol with the β_1-adrenoceptor.

Table 2. Slopes and pA_2 values from Schild plots for (±)-practolol and (±)-butoxamine vs (−)-noradrenaline and (±)-terbutaline.

	Agonist: (−)-Noradrenaline			(±)-Terbutaline		
Antagonist	Slope[a]	r[b]	pA_2[c]	Slope	r	pA_2
(±)-Practolol	0·70[d] ±0·06	0·92	6·32[e] (6·39, 6·25)	0·83 ±0·08	0·95	4·72 (4·78, 4·67)
(±)-Butoxamine	0·92 ±0·19	0·88	5·25 (5·36, 5·15)	1·04 ±0·16	0·84	5·92 (5·84, 6·00)

[a] Regression coefficient ± standard error calculated from data shown in Figure 2.
[b] Correlation coefficient; all were significant ($P < 0.05$).
[c] Mean and 95% confidence limits calculated from individual pA_2 values obtained from data in Fig. 2 using the equation, $pA_2 = \log (DR-1) - \log [I]$ (MacKay 1978). This method underestimates the variability of the pA_2 values since it ignores the variability in slope.
[d] Slope significantly less than 1·0 ($P < 0.01$); none of the other tabulated slopes were significantly different from 1·0.
[e] This value, as derived, does not provide a valid quantitative measure for characterizing the drug-receptor interaction.

DISCUSSION

Although the mean pA_2 value for practolol vs noradrenaline cannot be used to characterize quantitatively the receptor involved, it is obvious from Fig. 2 that practolol is more potent in antagonizing noradrenaline than terbutaline while butoxamine is opposite in potency relative to the two agonists. This is consistent with a mixed population of β_1- and β_2-adrenoceptor subtypes as proposed by Furchgott (1976).

The data in Table 2 demonstrate that Schild plots with slopes of 1 can be obtained for selective β-adrenoceptor antagonists in carbachol-contracted trachea. With the exception of practolol vs noradrenaline, these data differ from those of O'Donnell & Wanstall (1980) but are consistent with Furchgott's (1976) suggestion that the low slopes for selective antagonists are due to interaction of the agonists with more than one type of receptor. With three of the agonist-antagonist combinations such multiple interaction appears to have been avoided in the experiments reported here by pharmacologic isolation of the receptor-of-interest.

Thus, the slope of 1 of the Schild plot for butoxamine vs terbutaline is consistent with competitive interaction only at the β_2-adrenoceptor; presumably, any potential interaction at the β_1-adrenoceptor was prevented by practolol. Similarly, the Schild plot for butoxamine vs noradrenaline also had a slope of 1, suggesting competitive interaction at the

β_1-adrenoceptor. In this case, however, any potential crossover interaction at the β_2-adrenoceptor was presumably prevented by the high concentrations of butoxamine needed to study the interaction with its low-affinity, β_1, receptor (i.e. the concentrations ranged between the theoretical pA_{14} and pA_{84} for the β_2 receptor). This same reasoning can explain the results of practolol vs terbutaline and is supported by the close agreement between the pA_2 value in the present study (4·72) and the values reported for practolol by Furchgott (1976) vs isoprenaline and noradrenaline (both close to 4·75) in tracheal strips from a guinea-pig whose large isoprenaline to noradrenaline potency ratio suggested it had an almost exclusive β_2-adrenoceptor population.

Unlike the above agonist-antagonist combinations, the Schild plot for practolol vs noradrenaline in the presence of butoxamine failed to agree with the predicted value for a competitive antagonist acting at a single receptor. A plausible explanation for this failure lies in the relative potencies of the latter two agents at the β_2-adrenoceptor. While noradrenaline is a relatively selective β_1 agonist, it does interact with β_2-adrenoceptors at higher concentrations (Furchgott 1976). Butoxamine, on the other hand, is a β_2 antagonist of low potency ($K_B = 1{\cdot}2 \times 10^{-6}$ M, this study). It is possible, therefore, that at the higher concentrations of its CCRC, noradrenaline may have been able to compete successfully with 4×10^{-6} M butoxamine for the β_2-adrenoceptor.

If this assumption is valid, the present study shows that pharmacologic isolation of one β-adrenoceptor subtype, if adequately maintained, makes Schild plots with the predicted slope possible for selective antagonists in carbachol-contracted trachea; it does not explain why such isolation is necessary in this preparation but not, apparently, in intrinsic-tone trachea. O'Donnell & Wanstall were unable to explain the difference in slope they observed for selective antagonists between the two preparations. They suggested that if Furchgott's hypothesis was to explain the observations of their study, it was necessary to assume that the agonists act on both β_1- and β_2-adrenoceptors in carbachol-contracted trachea but only on one type of receptor in intrinsic-tone trachea. While the present study did not examine the latter question, it does show that if interaction of agonist and antagonist at one receptor only is assured, the Schild plots for selective antagonists obtained in carbachol-contracted trachea resemble those obtained in intrinsic-tone trachea. This suggests, indirectly, the possibility that for some unexplained reason only one receptor type is involved in β-adrenoceptor relaxation of intrinsic-tone trachea. This remains an interesting and provocative possibility for which direct experimental evidence is lacking.

In summary, the present study shows that Schild plots with slopes of 1 can be obtained in carbachol-contracted guinea-pig trachea for selective β-adrenoceptor antagonists if effective pharmacologic isolation of the receptor-of-interest is maintained by appropriate selective agonists and antagonists. These data support the hypothesis that slopes of less than 1 are due to interaction of agonists with more than one β-receptor subtype rather than the state of contraction of the trachea. The apparent failure to maintain adequate pharmacologic isolation in the case of practolol vs noradrenaline in the presence of butoxamine supports O'Donnell & Wanstall's suggestion that intrinsic-tone tracheal preparations are generally more suitable for obtaining pA_2 values of selective β-adrenoceptor antagonists for comparison with pA_2 values obtained in other tissues.

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