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Are the pA_2 values of selective β-adrenoceptor antagonists valid when obtained on guinea-pig tracheal preparations contracted with carbachol?

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On carbachol-contracted tracheal preparations from guinea-pigs, the slope of the Schild plot for propranolol (isoprenaline as agonist) was 1·0. The slope of the plots for atenolol (β_1-selective) and butoxamine (β_2-selective) were less than 1·0, whether isoprenaline or fenoterol was agonist. This was in contrast to previous reports on intrinsic tone tracheal preparations. It was established that the low slopes for atenolol and butoxamine on carbachol-contracted preparations were not related to aspects of the experimental procedures. Low slopes on carbachol-contracted preparations tended to occur when the antagonist used was selective and, although the reason for this is not clear, it may be related to the presence of both β_1- and β_2-adrenoceptors in this tissue. Therefore, it is suggested that pA_2 values obtained for selective β-adrenoceptor antagonists on guinea-pig tracheal preparations contracted with carbachol may not be strictly valid, and that antagonists should be studied on intrinsic tone tracheal preparations when pA_2 values are to be compared with pA_2 values from other tissues to quantify the selectivity of the antagonist.

pA_2 values are frequently obtained from plots of log molar antagonist concentration versus log (concentration ratio − 1) as proposed by Arunlakshana & Schild (1959). If a pA_2 value derived in this way is to be valid, the slope of the plot (referred to as a Schild plot) should theoretically be 1·0. However, pA_2 values quoted in the literature for β-adrenoceptor antagonists are often derived from data fitting Schild plots with slopes of less than 1·0. In various recent studies on guinea-pig tracheal preparations the slopes of the Schild plots for β-adrenoceptor antagonists were not significantly different from 1·0 if the preparations were used under intrinsic tone (Mylecharane & Raper 1973; O'Donnell et al 1980; O'Donnell & Wanstall 1979). In contrast, the slopes of the plots for some, but not all, antagonists were less than 1·0 if tracheal preparations were contracted with carbachol (Levy & Wilkenfeld 1970; Imbs et al 1977; O'Donnell et al 1980; O'Donnell & Wanstall, unpublished observations). The presence of carbachol should not, in theory, affect the slope or location of Schild plots for β-adrenoceptor antagonists on guinea-pig trachea (Ohashi 1976), provided that the experiments are carried out using the experimental conditions proposed by Furchgott (1972). Therefore, the literature on the slopes of Schild plots on carbachol-contracted preparations was re-examined and it was noted that slopes less than 1·0 occurred predominantly when the antagonist used was selective for β_1- or β_2-adrenoceptors. Thus, in the present study pA_2 values for a β_1-selective antagonist (atenolol) and a β_2-selective antagonist (butoxamine) have been obtained on carbachol-contracted trachea and the slopes of the Schild plots compared with those previously obtained on intrinsic tone trachea (O'Donnell & Wanstall 1979).

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MATERIALS AND METHODS

Tracheae were removed from female guinea-pigs (350–550 g) which had been pretreated with reserpine (1 or 5 mg kg^{-1} i.p.) 18–24 h before the experiments. Tracheal chain preparations were set up in Krebs solution aerated with 95% O_2; 5% CO_2 and at 37 °C and were contracted with 1 μM carbachol (O'Donnell et al 1980). This concentration of carbachol gave a submaximal contraction. Except where otherwise indicated in the Results, extraneuronal uptake and α-adrenoceptors were inhibited with 50 μM metanephrine and 10 μM phentolamine (both present in the Krebs solution for 30 min before and also throughout the experiment). When intrinsic tone preparations were used, these were set up as described by O'Donnell & Wanstall (1974).

Concentration-response curves to the β-adrenoceptor agonists were obtained in the absence and presence of increasing concentrations ([B]) of a β-adrenoceptor antagonist and EC50 values (concentration of agonist causing 50% of the maximum response to that agonist) determined. Consecutive antagonist concentrations on any one tissue differed by a factor of at least 3 and usually 10. The antag-

onist drug was in contact with the tissue for 60 min before commencing an agonist concentration-response curve. Each antagonist was examined at the same concentrations as had previously been used on intrinsic tone preparations (O'Donnell & Wanstall 1979). For each concentration of antagonist a value for concentration ratio (CR) was calculated by dividing the EC50 obtained in the presence of the antagonist by that obtained in the absence of the antagonist. These values of CR were corrected for any change in sensitivity to the agonist that was not due to the presence of the antagonist, using the appropriate correction factor. These correction factors were obtained in separate series of experiments, as described by O'Donnell & Wanstall (1979), and a correction factor was obtained for each of the agonist drugs under each different set of experimental conditions.

For each antagonist and set of conditions, log (corrected CR-1) was plotted against log [B] as proposed by Arunlakshana & Schild (1959) and a linear least squares regression analysis (Snedecor & Cochran 1967) used to obtain the line of best fit using the combined data from a number of animals.

Drugs and solutions

Atenolol (ICI); butoxamine hydrochloride (Burroughs Wellcome); carbachol (Sigma); fenoterol hydrobromide (Boehringer Ingelheim); (±)-isoprenaline sulphate (Sigma); metanephrine hydrochloride (Calbiochem); phentolamine methanesulphonate (Regitine, Ciba); (±)-propranolol hydrochloride (ICI); reserpine (Serpasil, Ciba). All drugs were obtained as pure powders except for phentolamine and reserpine which were obtained as solutions in ampoules. Stock solutions (10 or 100 mM) of atenolol, butoxamine, fenoterol, isoprenaline and metanephrine were made up in 0·01 M HCl and stock solutions of propranolol in distilled water. All dilutions were made in Krebs solution and kept ice-cold during the course of each experiment. The composition of the Krebs solution (mM) was: NaCl 114, KCl 4·7, $CaCl_2$ 2·5, KH_2PO_4 1·2, $MgSO_4$ 1·2, $NaHCO_3$ 25, glucose 11·7, ascorbic acid 1·1.

Statistical analyses

The significance of any deviation from unity of the slopes of the Schild plots has been calculated according to the methods described in Snedecor & Cochran (1967).

RESULTS AND DISCUSSION

The slopes of the Schild plots obtained for atenolol and butoxamine on carbachol-contracted tracheal preparations are shown in Table 1, together with the results obtained by O'Donnell & Wanstall (1979) on intrinsic tone tracheae. The slopes of the plots were significantly less than 1·0 on carbachol-contracted preparations but not on intrinsic tone preparations and the low slopes occurred with either isoprenaline or fenoterol as agonist. These results confirmed the occurrence of low slopes on carbachol-contracted trachea.

One reason for Schild plots having low slopes was suggested by Furchgott et al (1973) who demonstrated that, unless extraneuronal uptake was inhibited, the slope of the Schild plot for propranolol with isoprenaline as agonist was less than 1·0. Thus, the low slopes obtained in the present study might have been due to inadequate inhibition of extraneuronal uptake by metanephrine which was the inhibitor used in the carbachol-contracted preparations. This possibility was excluded since responses to fenoterol, unlike those to isoprenaline were not influenced by extraneuronal uptake

Table 1. Slopes (±s.e.) of Schild plots for atenolol and butoxamine, using isoprenaline or fenoterol as agonist, on guinea-pig intrinsic tone and carbachol-contracted tracheal preparations. Inhibitors of extraneuronal uptake and α-adrenoceptors were present (see Materials & Methods).

	Slopes ± s.e.			
	Atenolol		Butoxamine	
	Isoprenaline	Fenoterol	Isoprenaline	Fenoterol
Intrinsic tone trachea	0·96 ± 0·08† (9, 18)‡	0·91 ± 0·11† (7, 12)	0·87 ± 0·07† (14, 27)	1·03 ± 0·13† (6, 11)
Carbachol-contracted trachea	0·82 ± 0·06** (4, 16)	0·83 ± 0·05** (3, 12)	0·61 ± 0·06*** (4, 20)	0·60 ± 0·08*** (4, 15)

† Data from O'Donnell & Wanstall (1979).
‡ Number of animals, number of data points.
** Slope significantly less than 1·0 ($0·01 > P > 0·001$).
*** Slope significantly less than 1·0 ($P < 0·001$).

inhibition (O'Donnell & Wanstall 1976) and yet low slopes were obtained on carbachol-contracted trachea with fenoterol, as well as with isoprenaline, as agonist (Table 1).

The regime used to inhibit extraneuronal uptake and α-adrenoceptors on carbachol-contracted preparations (metanephrine and phentolamine) was different from that used previously on intrinsic tone preparations (phenoxybenzamine, O'Donnell & Wanstall 1979) for reasons discussed by O'Donnell & Wanstall (1977). Therefore, the experiments with butoxamine were repeated on both types of tracheal preparation without these inhibitors present. Fenoterol was used as the agonist because with this agonist it is not strictly necessary to inhibit either extraneuronal uptake (*vide supra*) or α-adrenoceptors (O'Donnell & Wanstall 1974). On each type of preparation the Schild plots with and without inhibitors were superimposed (Fig. 1). Therefore, at this stage we were confident that the inhibitor regimes used were not only adequate but also were not producing non-specific effects which might have accounted for the different slopes.

The other difference between the two types of tracheal preparation was the means used to achieve tone. On carbachol-contracted preparations the slope of the Schild plot could be low if the carbachol contraction progressively declined in magnitude during the course of an experiment. This is because the sensitivity of these preparations to β-adrenoceptor agonists is dependent on the level of contraction induced by the carbachol (Van den Brink 1973). However, neither atenolol nor butoxamine, even in the highest concentrations used, caused any reduction in the net response to the standard, submaximal concentration (1 μM) of carbachol used in the experiments (Fig. 2).

Another difference between the two types of preparation was that carbachol-contracted preparations were approximately 10 times less sensitive to both isoprenaline and fenoterol than intrinsic tone preparations. Therefore, in the presence of antagonists, isoprenaline or fenoterol were used on carbachol-contracted preparations in concentrations higher than any concentrations used on intrinsic tone preparations. It could be argued that these high agonist concentrations (1 μM and above) used on carbachol-contracted tracheae were causing non-specific relaxation of these tissues which accounted for the low slopes. However, this possibility could be discounted on the basis of results from a further series of experiments in which propranolol was examined on carbachol-contracted trachea using

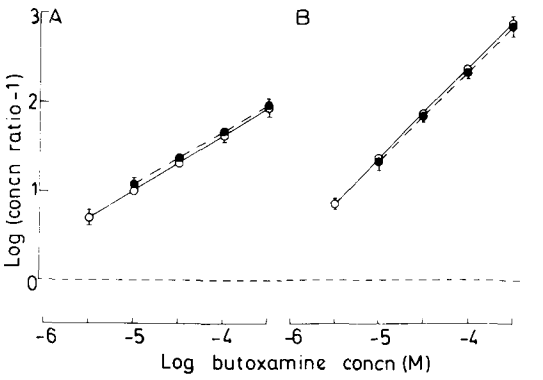

FIG. 1. Schild plots for butoxamine using fenoterol as agonist on guinea-pig isolated A, carbachol-contracted and B, intrinsic tone tracheal preparations. Inhibitors of extraneuronal uptake and α-adrenoceptors were absent (○—○) or present (●- - -●). The lines represent lines of best fit for the combined data from the number of animals and points shown in Table 1 and below. These lines were calculated using a linear least squares regression analysis of y on x and the estimates of y corresponding to the antagonist concentrations used in each group of experiments are shown. Vertical bars represent the standard errors of these estimated y values. The slopes of the plots in the absence of inhibitors were intrinsic tone: 1·03 ± 0·06 (7, 13) and carbachol-contracted 0·62 ± 0·08 (6, 12). Slopes with inhibitors are shown in Table 1. The slopes of the Schild plots were less than 1·0 on carbachol-contracted preparations and omission of the inhibitors did not alter the location or slopes of the Schild plots.

isoprenaline as agonist. In these experiments some doses of isoprenaline used on carbachol-contracted preparations were again higher than those ever used on intrinsic tone preparations yet the slope of the Schild plot for propranolol was *not* less than 1·0. The slope value (1·05 ± 0·14; 4 animals, 15 data points) was not different from the value for propranolol obtained by O'Donnell & Wanstall (1979) on intrinsic tone preparations (0·96 ± 0·10).

Since the findings with propranolol differed from those with atenolol or butoxamine, it seemed that the low slopes on the carbachol-contracted preparations were not related to the nature or design of the experiments but rather reflected some property of the antagonist drugs. Thus, a theoretical explanation for the results was sought based on the observation, from this and other studies, that the slopes of the Schild plots on carbachol-contracted preparations were low for the selective antagonists atenolol (this study), butoxamine (this study; Levy & Wilkenfeld 1970; Imbs et al 1977), α-methylpropranolol (O'Donnell et al 1980) practolol (Levy & Wilkenfeld, 1970; Imbs et al 1977) and H35/25 (O'Donnell &

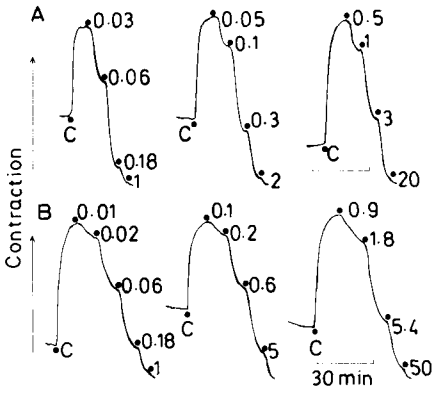

FIG. 2. Shows two preparations of guinea-pig carbachol-contracted tracheal chain preparations. Metanephrine (50 μM) and phentolamine (10 μM) were present to inhibit extraneuronal uptake and α-adrenoceptors respectively. At C 1 μM carbachol was added. On preparation A concentration-response curves to isoprenaline are shown (all concentrations are in μM) before and then 60 min after contact firstly with 3 μM and then with 300 μM butoxamine. On preparation B concentration-response curves to fenoterol are shown before and 60 min after contact firstly with 30 μM and then with 300 μM atenolol. The level of the carbachol contraction was not affected by either butoxamine or atenolol. Time bracket: 30 min.

Wanstall unpublished observations) but not for the non-selective antagonists propranolol (this study; Furchgott et al 1973), 4BIP (O'Donnell et al 1980) and sotalol (Buckner et al 1974). Furchgott (1976) has postulated that, if a tissue has two receptor types then the slope of the Schild plot for an antagonist which is selective for one or other of the receptor types will be less than 1·0 if the agonist used acts on both receptors, but equal to 1·0 if the agonist acts on only one of the receptors. Guinea-pig trachea is now believed to possess a mixture of β_1- and β_2-adrenoceptors (Furchgott 1976; O'Donnell & Wanstall 1979). However, if the hypothesis put forward by Furchgott (1976) is to explain the observations made in the present study, it is necessary to assume that the agonists act on both β_1- and β_2-adrenoceptors in carbachol-contracted preparations giving slopes of less than 1·0, but on only β_2- (or β_1)-adrenoceptors in intrinsic tone preparations, giving slopes of 1·0. The necessary experimental evidence to support this assumption is not available at present.

In summary, although the differences between the two types of tracheal preparation reported in this paper remain unexplained, one conclusion of practical importance can be drawn from the findings. It would appear that intrinsic tone preparations are more suitable than carbachol-contracted preparations for determining the pA_2 values of selective β-adrenoceptor antagonists because Schild plots with slopes of 1·0 can be obtained. A slope of 1·0 is particularly important if pA_2 values on trachea are to be compared with pA_2 values on some other tissue, e.g. atria, in order to quantify the selectivity of an antagonist. Comparison of pA_2 values on two different tissues is not valid unless both pA_2 values were obtained from data which yielded Schild plots with slopes of 1·0.

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REFERENCES

Arunlakshana, O., Schild, H. O. (1959) Br. J. Pharmacol. Chemother. 14: 48–58

Buckner, C. K., Birnbaum, J., O'Connor, M. (1974) Eur. J. Pharmacol. 26: 198–203

Furchgott, R. F. (1972) in: Blaschko, H., Muscholl, E. (eds) Handbook of Experimental Pharmacology. Springer New York, 33, pp 283–335

Furchgott, R. F. (1976) in: Bevan, J. A. (ed) Vascular Neuroeffector Mechanisms. 2nd Int. Symp. Odense, Karger Basel, pp 131–142

Furchgott, R. F., Jurkiewicz, A., Jurkiewicz, N. H. (1973) in: Usdin, E., Snyder, S. H. (eds) Frontiers in catecholamine research. Pergamon Press, New York, pp 295–299

Imbs, J. L., Miesch, F., Schwartz, J., Velly, J., Leclerc, G., Mann, A., Wermuth, C. G. (1977) Br. J. Pharmacol. 60: 357–362

Levy, B., Wilkenfeld, B. E. (1970) Eur. J. Pharmacol. 11: 67–74

Mylecharane, E. J., Raper, C. (1973) Ibid. 21: 375–378

O'Donnell, S. R., Walduck, K., Wanstall, J. C. (1980) Br. J. Pharmacol. 68: 705–710

O'Donnell, S. R., Wanstall, J. C. (1974) Ibid. 52: 407–417

O'Donnell, S. R., Wanstall, J. C. (1976) Ibid. 57: 369–373

O'Donnell, S. R., Wanstall, J. C. (1977) Ibid. 60: 255–262

O'Donnell, S. R., Wanstall, J. C. (1979) Naunyn-Schmiedeberg's Arch. Pharmacol. 308: 183–190

Ohashi, M. (1976) Jpn. J. Pharmacol. 26: 325–330

Snedecor, G. W., Cochran, W. G. (1967) Statistical Methods 6th ed. The Iowa State University Press. Iowa, pp 137–171

Van den Brink, F. G. (1973) Eur. J. Pharmacol. 22: 279–286