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How β_2-selective is the adrenoceptor antagonist drug, IPS 339?

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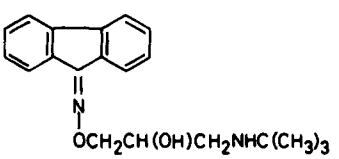
Schild plots for the compound (t-butylamino-3-ol-2-propyl)oximino-9-fluorene (IPS 339) have been obtained on isolated intrinsic tone trachea and atria (rate) of guinea-pigs. α-Adrenoceptors and uptakes were inhibited. The Schild plots for IPS 339 on trachea (fenoterol as agonist) and on atria (noradrenaline as agonist) were not superimposed suggesting that IPS 339 was β_2-selective. The slopes of the Schild plots obtained on intrinsic tone tracheal preparations (isoprenaline or fenoterol as agonist), although greater than 1·0, were not significantly different from that on atria (noradrenaline as agonist). From the average separation of these Schild plots on trachea and atria IPS 339 was assessed to be only 3·3 times more active on β_2- than on β_1-adrenoceptors. The experiments in the literature which showed a high β_2-selectivity for IPS 339 (155 fold) were carried out on carbachol-contracted tracheal preparations (isoprenaline as agonist) and the Schild plot obtained had a very low slope which was quite different from that on atria. Therefore, the results illustrate how the quantitative estimate of the selectivity of a β-adrenoceptor antagonist can be misleading when Schild plots with different slopes are compared.

The compound (t-butylamino-3-ol-2-propyl)oximino-9-fluorene (IPS 339 I), was first described by Imbs et al (1977). These workers concluded from pA_2 data that this compound was a potent, β_2-selective adrenoceptor antagonist and it has been used as such by other groups of workers (Ek & Lundgren 1979; Minneman et al 1979; Himori et al 1979). However, because the experimental conditions employed by Imbs et al (1977) were not optimal, e.g. uptake and α-adrenoceptors were not

I.



blocked, and because the slope of the Schild plot on the carbachol-contracted tracheal preparations which they used was less than the theoretical value of unity, we have re-examined this compound, as part of a study on the β_2-selectivity of adrenoceptor antagonists (O'Donnell & Wanstall 1979; O'Donnell et al 1980). The results suggest that, whilst IPS 339 shows some β_2-selectivity, it is not as selective as initially suggested by Imbs et al (1977).

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METHODS

Female guinea-pigs (400–500 g), pretreated with reserpine (1 mg kg^{-1} i.p. 18–24 h before the experiment), were killed by a blow on the head and the trachea and atria removed. Intrinsic tone tracheal preparations and spontaneously beating atrial preparations were set up as described by O'Donnell & Wanstall (1979), i.e. phenoxybenzamine was used to block neuronal and extraneuronal uptake mechanisms and α-adrenoceptors, and all experiments were carried out at 37 °C in Krebs solution aerated with 95% O_2, 5% CO_2.

Tracheal relaxation and atrial rate were measured. Agonist concentration-response curves were obtained in the absence and presence of various concentrations of IPS 339. One or two, or occasionally three, different concentrations were examined on each preparation. Concentration-ratios were corrected for spontaneous changes in sensitivity of the preparations and then used to obtain Schild plots and pA_2 values as described by O'Donnell & Wanstall (1979).

Drugs used: (t-butylamino-3-ol-2-propyl)oximino-9-fluorene as acetate (gift from Dr H. Tucker, ICI, U.K.) and as hydrochloride (code named IPS 339, gift from Dr J. L. Imbs, France), fenoterol hydrobromide (Boehringer Ingelheim), isoprenaline sul-

phate (Burroughs Wellcome), noradrenaline acid tartrate (Astra), phenoxybenzamine hydrochloride (Smith, Kline and French) and reserpine (Serpasil, Ciba). All drugs were used as pure powders, except for reserpine, which was obtained as a solution in ampoules. Isoprenaline, fenoterol and noradrenaline were made up in 0·1 M HCl to give stock solutions of 10 or 100 mM. A fresh stock solution of 1 mM (t-butylamino-3-ol-2-propyl)oximino-9-fluorene (acetate or hydrochloride) in 0·01 M HCl was prepared each day. Dilutions were made in Krebs solution immediately before use and the solutions kept on ice for the duration of the experiment. The Krebs solution used contained (mM): NaCl 114, KCl 4·7, $CaCl_2$ 2·5, KH_2PO_4 1·2, $MgSO_4$ 1·2, $NaHCO_3$ 25, glucose 11·7 and ascorbic acid 1·1. It was aerated with 95% O_2 and 5% CO_2.

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

It was intended to use the approach of O'Donnell & Wanstall (1979) to estimate the β_2-selectivity of IPS 339 i.e. to obtain the pA_2 values for IPS 339 on trachea with fenoterol as agonist and on atria with noradrenaline as agonist. The Schild plots for these data are shown in Fig. 1A. These plots represent pooled results from two series of experiments, one carried out with IPS 339 acetate provided by ICI (U.K.) and the other carried out with IPS 339 hydrochloride, provided by the original workers (Imbs et al 1977). The data were combined since there was no significant difference between the Schild plot regression lines obtained with either material. The slopes of these Schild plots were greater than unity, although this was not statistically significant on atria (Table 1). Therefore, calculation of pA_2 values, either by extrapolation of the Schild plots or by applying the formula $pA_2 = \log(CR - 1) - \log[B]$, which assumes that the slope is 1·0 (O'Donnell & Wanstall 1979), is not strictly valid. Nevertheless, the Schild plots on trachea and atria in Fig. 1A are not superimposed suggesting that IPS 339 caused more antagonism of responses mediated via β_2-adrenoceptor stimulation in trachea than via β_1-adrenoceptor stimulation in atria. Furthermore, since the slopes of the Schild plots on trachea and atria shown in Fig. 1A were not significantly different from each other, the 0·52 log unit average separation of these two lines suggests that the β_2-selectivity of IPS 339 is only 3·3. This is much less than the 155 fold reported by Imbs et al (1977).

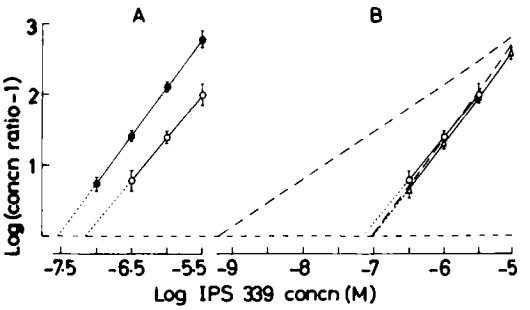

FIG. 1. Schild plots for IPS 339 on guinea-pig intrinsic tone tracheal preparations and atrial preparations. Data from the present study with fenoterol as agonist on trachea (●–●) and with noradrenaline as agonist on atria (○–○) are shown in A. These lines represent the lines of best fit through the combined data from the number of animals and points shown in Table 1. The lines were calculated by a linear least squares regression analysis of y on x and the estimates of y for the antagonist concentrations used are shown with standard error bars. Also shown in B are the locations of the plots for isoprenaline on trachea (T) and atria (A) calculated from the pA_2 values and slope data given by Imbs et al (1977). These plots are the broken lines. Also shown are the plots on intrinsic tone trachea with isoprenaline as agonist (△–△) and on atria with noradrenaline as agonist (○–○) from the present study.

For comparison with the results of Imbs et al (1977), the values for pA_2 obtained from the present data, either by extrapolation of the Schild plots or by calculation, are shown in Table 1. The atria results with noradrenaline as agonist (Table 1) are very close to the results of Imbs et al (1977) with isoprenaline as agonist (pA_2 7·04, slope of Schild plot 1·32 ± 0·25, Fig. 1B). Since O'Donnell &

Table 1. Slopes of the Schild plots for IPS 339, using various agonists on trachea and atria, together with extrapolated pA_2 values and mean pA_2 ± s.e. by calculation.

Tissue	Agonist	Slope of Schild plot ± s.e. of slope	pA_2 from Schild plot†	pA_2 ± s.e.‡
Atria	Noradrenaline	1·21 ± 0·23 (22)*a*	7·15	7·29 ± 0·09 (13)*b*
Intrinsic tone trachea	Fenoterol	1·37 ± 0·12* (27)	7·54	7·98 ± 0·09 (14)
Intrinsic tone trachea	Isoprenaline	1·29 ± 0·11* (25)	7·00	7·36 ± 0·07 (13)

a Number of points.
b Number of animals.
† Obtained by extrapolation.
‡ Calculated by the method of O'Donnell & Wanstall (1979).
* Slope significantly greater than unity $0·05 > P > 0·01$.

Wanstall (1979) have previously shown that the pA_2 of selective β-adrenoceptor antagonists should be independent of the agonist used on guinea-pig atria, the closeness of the pA_2 values was indicative that differences between the two laboratories were not due to the atrial results. In contrast, the discrepancies were related to the results on trachea.

The pA_2 value quoted by Imbs et al (1977) on trachea was obtained on carbachol-contracted preparations with isoprenaline as agonist. The slope quoted (0·66) was significantly less than unity and different from that on atria (1·32). O'Donnell & Wanstall (1979, 1980a) have recently shown that the location of Schild plots on guinea-pig trachea depends on the agonist used and also that selective β-adrenoceptor antagonists tend to have Schild plots with a slope less than 1·0 on guinea-pig carbachol-contracted trachea but not intrinsic tone trachea. Thus another series of experiments were carried out with IPS 339 using isoprenaline, the agonist used by Imbs et al (1977), but on intrinsic tone trachea. The Schild plot for these results is shown in Fig. 1B together with the location of the plot predicted from the published data of Imbs et al (1977) on trachea. The results illustrate the reason for the high estimate of the selectivity of IPS 339 in the experiments of Imbs et al (1977) and confirm the suggestion of O'Donnell & Wanstall (1980a) that pA_2 values for selective β-adrenoceptor antagonists should be obtained on intrinsic tone tracheal preparations if they are to be compared with pA_2 values from other tissues to quantify the selectivity of an antagonist. Recently, a pA_2 value for IPS 339 was quoted from results on histamine-contracted trachea (Holmberg et al 1980) and again the value (8·84) is not valid because the slope of the Schild plot on trachea was much less than unity (0·55).

It remains to be explained why the slopes of the Schild plots in this study were all greater than unity. On trachea, IPS 339 caused a parallel shift in the log concentration-response lines to both fenoterol and isoprenaline and caused no other marked effects on the preparations. On atria, IPS 339 caused a parallel shift in the log concentration-response lines to noradrenaline. It caused some depression of maximum atrial rate at the highest concentration used (3×10^{-6} M) but, since there was also some depression of resting rate, the maximum *increase* in rate was not affected. IPS 339 in a concentration of 1×10^{-5} M frequently caused the atria to stop beating and so this concentration was not used. One could speculate that IPS 339 is not a simple competitive β-adrenoceptor antagonist, but also has a non-competitive component in its action.

In summary, the compound IPS 339 does show β_2-selectivity in vitro when examined on guinea-pig tracheal and atrial preparations but it is not as selective as was reported by Imbs et al (1977). The reason for the discrepancy between the present results and those of Imbs et al (1977) relates to the use by them of data obtained on carbachol-contracted guinea-pig trachea. The present study has reinforced the previous conclusion (O'Donnell et al 1980; O'Donnell & Wanstall 1980a) that anomalies between results for selective β-adrenoceptor antagonists from different laboratories frequently involve the use of pA_2 values obtained on carbachol-contracted tracheal preparations. These can give Schild plots with slopes less than unity. The compound IPS 339 in our studies has been shown to be less β_2-selective than butoxamine, H 35/25, α-methylpropranolol (O'Donnell & Wanstall 1979) or ICI 118,551 (O'Donnell & Wanstall 1980b) under identical experimental conditions.

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REFERENCES

Ek, B., Lundgren, B. (1979) Acta Physiol. Scand. Suppl. 473: 43

Himori, N., Hayakawa, S., Ishimori, T. (1979) Life Sci. 24: 1953–1958

Holmberg, E., Jeppsson, A.-B., Lamm, C. J., Waldeck, B. (1980) Acta Pharmacol. Toxicol. 46 : 150–155

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

Minneman, K. P., Hedberg, A., Molinoff, P. B. (1979) J. Pharmacol. Exp. Ther. 211: 502–508

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

O'Donnell, S. R., Wanstall, J. C. (1980a) J. Pharm. Pharmacol. 32: 413–416

O'Donnell, S. R., Wanstall, J. C. (1980b) Life Sci. 27: 671–677

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

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