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A STUDY OF SOME PROPRANOLOL ANALOGUES FOR SELECTIVE β-ADRENOCEPTOR ANTAGONISM USING pA_2 VALUES ON ISOLATED TRACHEA AND ATRIA FROM GUINEA-PIG

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1 pA_2 values for β-adrenoceptor antagonists were obtained on isolated preparations of guinea-pig trachea (intrinsic tone) and atria (rate), with isoprenaline, noradrenaline (β_1-selective) and fenoterol (β_2-selective) as agonists. Uptake mechanisms and α-adrenoceptors were inhibited. The antagonists studied were (±)-threo-α-methylpropranolol, (±)-1-(4-benzimidazoloxy)-3-isopropylamino-2-propanol (4-BIP) and (±)-1-(5-benzimidazoloxy)-3-isopropylamino-2-propanol (5-BIP).

2 4-BIP was a potent β-adrenoceptor antagonist but it was not selective for trachea (pA_2 on trachea 7.88 and on atria 7.73, fenoterol as agonist). 5-BIP was less than one tenth as active as 4-BIP and was therefore not studied in detail.

3 α-Methylpropranolol was potent and it was also selective for trachea (pA_2 on trachea 8.24 and on atria 7.56, fenoterol as agonist). This selectivity was not seen with isoprenaline as agonist. In tracheal preparations contracted by carbachol the slope of the Schild plot for α-methylpropranolol was less than 1.0 (isoprenaline as agonist).

4 α-Methylpropranolol, although not highly selective for β_2-adrenoceptors, is considerably more potent than the alternative β_2-selective antagonists available at present. Therefore, it may be useful in studies designed to classify β-adrenoceptor subtypes in tissues.

Introduction

At present there is no potent antagonist that is selective for β_2-adrenoceptors. Butoxamine and H35/25 [1-(4'-methylphenyl)-2-isopropylaminopropanol] are reasonably selective but both antagonists are weak (Wanstall, O'Donnell & Walduck, 1979), which severely limits their usefulness in isolated tissue experiments.

This paper describes results obtained with three analogues of propranolol on isolated preparations of guinea-pig trachea and atria in which α-adrenoceptors and uptake mechanisms were blocked as recommended by Furchgott (1972). pA_2 values for the antagonists have been obtained with three different β-adrenoceptor agonists. Two of the antagonists examined were benzimidazole analogues of propranolol (Figure 1), the synthesis of which has been recently described (Fauland, Kampe, Thiel, Bartsch & Schaumann, 1976; Crooks, Wright, Callery & Moreton, 1979). Crooks and his co-workers suggested that one of these compounds, which they referred to as 4-BIP (±)-1-(4-benzimidazoloxy)-3-isopropylamino-2-propanol (Figure 1), was a potent selective β_2-adrenoceptor antagonist which they deduced from

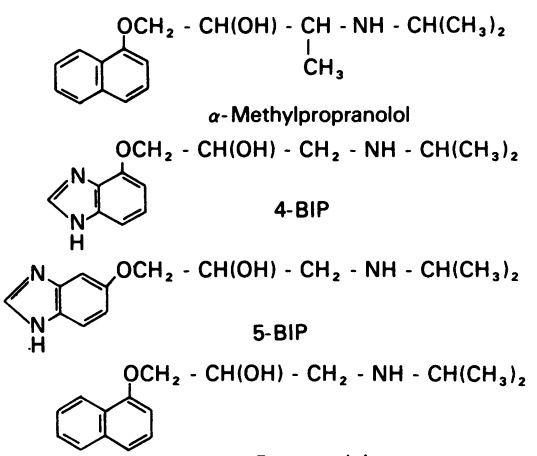

Figure 1 Chemical structures of the three propranolol analogues studied: (±)-threo-α-methylpropranolol (α-methylpropranolol), (±)-1-(4-benzimidazoloxy)-3-isopropylamino-2-propanol (4-BIP); (±)-1-(5-benzimidazoloxy)-3-isopropylamino-2-propanol (5-BIP) and the parent compound, propranolol.

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pA_2 values on trachea and atria with isoprenaline as agonist. The third compound examined was α-methylpropranolol (Figure 1), a compound described as selective for β_2-adrenoceptors on the basis of *in vivo* experiments (Levy, 1973; Todd, 1976; Fitzgerald & O'Donnell, 1978). In the present study an attempt has been made to quantify the selectivity of this antagonist *in vitro*.

A preliminary account of this work was presented to the 12th meeting of the Australasian Society of Clinical and Experimental Pharmacologists (O'Donnell, Walduck & Wanstall, 1979).

Methods

Female guinea-pigs (350 to 500 g), pretreated with reserpine (5 mg/kg, intraperitoneally, 18 to 24 h previously) were used. Intrinsic tone tracheal chain preparations (O'Donnell & Wanstall, 1974) were set up at a resting tension of 500 mg and allowed to gain tone. For these preparations and for the spontaneously beating atrial preparations from the same animals, phenoxybenzamine (50 μM for 30 min followed by a 30 min period of thorough washing) was used to block neuronal and extraneuronal uptake mechanisms and α-adrenoceptors. In some experiments tracheal chains were contracted with carbachol (1 μM). These were also set up at a resting tension of 500 mg but for these preparations, and for the atria from the same animals, metanephrine (50 μM) and phentolamine (10 μM) were present in the Krebs solution for 30 min before, and also during the determination of an agonist concentration-response curve, instead of phenoxybenzamine. The reasons for the different regimes to block α-adrenoceptors and extraneuronal uptake are discussed by O'Donnell & Wanstall (1977). All experiments were carried out at 37°C. Relaxation responses of tracheal preparations to agonist drugs were recorded isotonically with a modified Statham 10B strain gauge and atrial rate was measured with a pulse counter triggered by the interruption of a light beam. All agonist drug additions were made by the cumulative method; an Agla micrometer syringe was used to add small volumes. At the end of each cumulative concentration-response curve the maximum response to the relevant agonist was routinely determined. The contact time of 1 h selected for the β-adrenoceptor antagonists was shown to produce equilibrium blockade.

Treatment of data

pA_2 values for the antagonist drugs were obtained from Schild plots of log molar concentration antagonist against log (concentration ratio -1) as described by Arunlakshana & Schild (1959). Values for concentration ratio were obtained from EC_{50} values (concentrations producing 50% maximum response to the agonist in that concentration-response curve) in the presence and absence of antagonist. A linear least squares regression analysis was used to obtain the line of best fit using points obtained from a number of animals. The slope ± s.e. slope of this regression line was obtained by methods described by Snedecor & Cochran (1967).

Because selectivity depends on a comparison of accurate pA_2 values between two different tissues, experiments were carried out to determine whether tracheal or atrial preparations changed in sensitivity to the agonists during the time required to obtain data for the pA_2 values. If sensitivity does change, Furchgott (1972) recommends that concentration ratios be corrected to compensate for this, as pA_2 values could otherwise be affected. Since atrial preparations and trachea contracted by carbachol did not vary in sensitivity to isoprenaline, correction of these data was not necessary. Intrinsic tone tracheal preparations increased in sensitivity to all three agonists and atrial preparations decreased in sensitivity to noradrenaline and fenoterol. Therefore the corresponding concentration ratios were adjusted by use of correction factors obtained from at least five control experiments in the absence of antagonists.

Drugs and solutions used

Drugs used were: 4-BIP hydrochloride and (±)-1-(5-benzimidazoloxy)-3-isopropylamino-2-propanol (5-BIP) hydrochloride (gift from Dr J. Wright), carbachol (Sigma), fenoterol hydrobromide (gift from Boehringer Ingelheim), (±)-isoprenaline sulphate (Burroughs–Wellcome), metanephrine hydrochloride (Calbiochem), α-methylpropranolol hydrochloride (gift from ICI), noradrenaline acid tartrate (gift from Astra), pargyline hydrochloride (Sigma), phenoxybenzamine hydrochloride (Smith, Kline & French), phentolamine methanesulphonate (Regitine, Ciba) and reserpine (Serpasil, Ciba). All drugs were used as pure powders except for reserpine and phentolamine, which were obtained as solutions in ampoules. Isoprenaline, fenoterol, noradrenaline, 4-BIP and 5-BIP were made up in 0.01 M HCl to give stock solutions of 10 or 100 mM. A fresh stock solution of 10 mM α-methylpropranolol in deionized water was prepared each day. Dilutions were made in Krebs solution immediately before the experiment and the solutions kept on ice for the duration of the experiment.

The Krebs solution 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.

Results

Experiments were initially carried out on atrial and intrinsic-tone tracheal chain preparations to ascertain whether an inhibitor of monoamine oxidase was necessary, in addition to neuronal and extraneuronal uptake inhibitors, when using noradrenaline as agonist. Pargyline (500 μM, 15 min contact followed by 15 min washout) caused no further potentiation of responses to noradrenaline after treatment of preparations with phenoxybenzamine (Figure 2). Thus, a monoamine oxidase inhibitor was not included in experiments with noradrenaline.

pA_2 values and selectivity of antagonists

The pA_2 values and slopes of the Schild plots obtained for α-methylpropranolol and 4-BIP on atria and intrinsic tone trachea are shown in Table 1, together with selectivity values. For α-methylpropranolol the pA_2 value on trachea varied with the agonist used, being greatest with fenoterol and least with noradrenaline (Figure 3). For 4-BIP, the pA_2 value on trachea did not vary with the agonist and the Schild plots were virtually superimposable (Figure 3). The choice of agonist drug had no influence on the pA_2 for either α-methylpropranolol or 4-BIP on atria (Figure 3). None of the slopes differed significantly

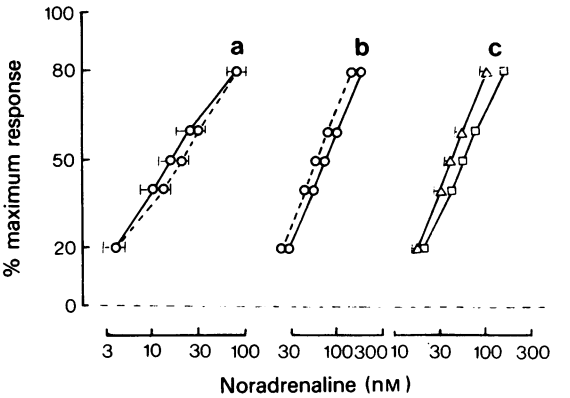

Figure 2 Mean concentration-response curves to noradrenaline on atrial preparations (a) and paired intrinsic tone tracheal chain preparations (b,c) from 5 guinea-pigs. On the atrial preparations and on one of the tracheal chain preparations a concentration-response curve was obtained before (O——O) and after (O- - -O) the addition of pargyline (500 μM, 15 min contact followed by 15 min wash). On the other tracheal preparation the concentration-response curve to noradrenaline was repeated without pargyline present (□——□ 1st line, △——△ 2nd line). Mean curves were plotted by the method of Langer & Trendelenburg (1969) and horizontal lines represent s.e. mean.

Table 1 Trachea: atria selectivity values, pA_2 values and slopes (±s.e. slope) of Schild plots for α-methylpropranolol and (±)-1-(4-benzimidazoloxy)-3-isopropylamino-2-propanol (4-BIP) on trachea (intrinsic tone) and on atria (rate) with isoprenaline, noradrenaline and fenoterol as agonists

Antagonist drug	*Tissue*	*pA_2 values of antagonist drug* *Isoprenaline as agonist*	*Noradrenaline as agonist*	*Fenoterol as agonist*
α-Methylpropranolol	Trachea	7.71 (16, 32)‡ [1.11 ± 0.08]†	7.45 (8, 16) [0.96 ± 0.07]	8.24 (8.16) [1.15 ± 0.14]
	Atria	7.49 (5, 14) [1.05 ± 0.05]	7.48 (8, 16) [1.01 ± 0.09]	7.56 (7,14) [0.90 ± 0.08]
	Trachea:atria selectivity*	1.7	0.93	4.8
4-BIP	Trachea	7.79 (4, 12) [0.97 ± 0.02]	7.73 (4, 12) [1.01 ± 0.03]	7.88 (4, 12) [1.07 ± 0.09]
	Atria	7.53 (4, 12) [1.07 ± 0.06]	7.55 (4, 12) [1.04 ± 0.04]	7.73 (4, 12) [0.96 ± 0.10]
	Trachea:atria selectivity	1.8	1.5	1.4

Tissues were treated with phenoxybenzamine (50 μM) for 30 min, followed by wash out, before the experiment. The Schild plots and pA_2 values were based on concentration-ratios which had been corrected for changes in sensitivity of the tissues.

* Antilog (pA_2 trachea − pA_2 atria).

† Slope of regression line ± s.e. slope.

‡ Number of animals and points, respectively, used in linear least squares regression analysis.

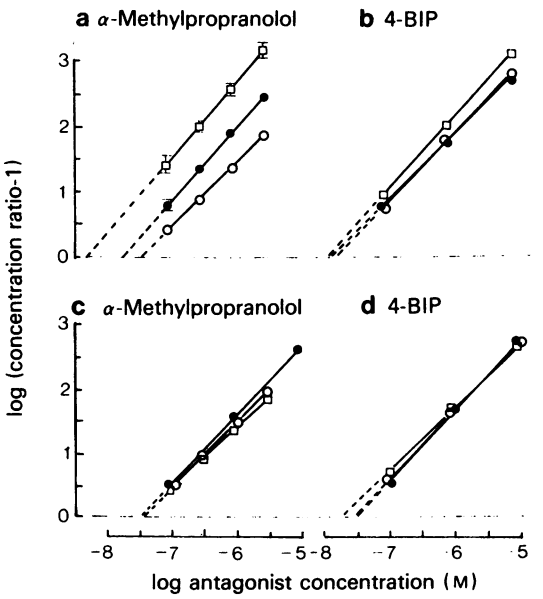

Figure 3 Schild plots for the antagonism of noradrenaline (○), isoprenaline (●) and fenoterol (□) on guinea-pig intrinsic tone tracheal chain preparations (upper graphs) and atrial preparations (lower graphs) by (a) α-methylpropranolol and (b) (±)-1-(4-benzimidazoloxy)-3-isopropylamino-2-propanol (4-BIP). The concentration-ratios were corrected for spontaneous sensitivity changes and the lines represent the lines of best fit for the combined data from the number of animals and points shown in Table 2. The lines were calculated using a linear least squares regression of y on x and points have been put on this line at the estimates of y for the antagonist concentrations used in each group of experiments. The vertical lines represent the s.e. of these estimated y values.

from 1.0 (Table 1) and hence pA_2 values could justifiably be compared to provide the selectivity values. With fenoterol as agonist α-methylpropranolol was selective for trachea (4.8-fold) whereas 4-BIP was not (1.4-fold) (Table 1). If isoprenaline or noradrenaline was the agonist, neither α-methylpropranolol nor 4-BIP was selective (<2-fold).

The results of further experiments carried out on the type of tracheal preparation frequently used by other workers, i.e. carbachol-contracted, are summarised in Table 2, together with the data from the paired atrial preparations. The results obtained on atria were very close to those shown in Table 1, despite the different inhibitor regime and the use of corrected concentration-ratios in the former experiments. For α-methylpropranolol, the Schild plot on trachea had a slope which was significantly less than 1.0 and significantly less than that on atria. Hence the pA_2 values could not justifiably be compared nor selectivity be assessed. 5-BIP was less than one tenth as active as the other two compounds.

Discussion

α-Methylpropranolol showed greater potency on trachea than on atria provided fenoterol was agonist (pA_2 on trachea 8.24 and on atria 7.56) i.e. it was selective for trachea. This suggests that α-methylpropranolol is selective for β_2-adrenoceptors.

However, the pA_2 values for α-methylpropranolol on guinea-pig trachea varied according to which agonist was employed (with isoprenaline the pA_2 value was 7.71 and with noradrenaline 7.45). Such a variation might be expected if a tissue possesses more than one type of receptor and if (a) the agonists differ from one another in their selectivity for the different

Table 2 pA_2 values and slopes (±s.e. slope) of Schild plots for α-methylpropranolol, (±)-1-(4-benzimidazoloxy)-3-isopropylamino-2-propanol (4-BIP) and 5-BIP on trachea (carbachol-contracted, 1 μM) and on atria with isoprenaline as agonist

	pA_2 values for antagonist drug		
Tissue	*α-Methylpropranolol*	*4-BIP*	*5-BIP*
Trachea	7.96 (8, 22)‡	7.25 (5, 20)	6.26 (6, 17)
	[0.82 ± 0.07]†*	[0.98 ± 0.09]	[0.91 ± 0.04]*
Atria	7.31 (9, 15)	7.51 (4, 11)	6.05 (6, 17)
	[1.22 ± 0.08]*	[1.04 ± 0.04]	[0.97 ± 0.02]

Metanephrine (50 μM) and phentolamine (10 μM) were present in the Krebs solution.
* Slope significantly different from 1.0 ($0.05 > P > 0.01$).
† Slope of regression line ± s.e. slope.
‡ Number of animals and points respectively used in linear least squares regression analysis.

receptors and (b) the antagonist is selective for one of the receptors. Thus, the measured pA_2 value of an antagonist selective for β_2-adrenoceptors against a non-selective agonist, such as isoprenaline, will not provide an accurate estimate of the potency of the antagonist on β_2-adrenoceptors. Indeed, α-methylpropranolol would have been adjudged 'non-selective' if isoprenaline had been the only agonist used in these studies (pA_2 on trachea 7.71 and on atria 7.49). These findings emphasize the point that a β_2-selective agonist, such as fenoterol in the present study, should be used to assess the selectivity of an antagonist at β_2-adrenoceptors in tissues like trachea with mixed receptor populations. However, in tissues like guinea-pig atria which contain only one receptor type (O'Donnell & Wanstall, 1979) the degree of selectivity of the agonist is immaterial, supported by the present findings that pA_2 values on guinea-pig atria did not vary with different agonists.

Comparison of the data for α-methylpropranolol with the results for propranolol from similar experiments (O'Donnell & Wanstall, unpublished results) showed that insertion of the α-methyl group into the propranolol molecule did enhance the selectivity for β_2-adrenoceptors. Maybe the reason that this was not detected previously *in vivo* was because isoprenaline was used as the agonist (O'Donnell & Walduck, 1978; Fitzgerald & O'Donnell, 1978).

In contrast to α-methylpropranolol, no evidence was obtained to suggest that 4-BIP was selective for β_2-adrenoceptors, as shown by pA_2 values with fenoterol as agonist (pA_2 on trachea 7.88 and on atria 7.73) and by the constancy of pA_2 values on trachea with different agonists. The compound 5-BIP was not investigated in detail because of its relatively low potency compared with α-methylpropranolol and 4-BIP.

The conclusion that 4-BIP was not selective differed from that of Crooks *et al.* (1979) whose data indicated that the drug was 17.4 times more potent on trachea than on atria. The anomaly between the two laboratories was not related to the atrial results. Two estimates for the pA_2 of 4-BIP on atria, with isoprenaline as agonist, were obtained under two different sets of conditions and the values were very close to one another (7.51 and 7.53) and also to the value quoted by Crooks *et al.* (1979) on atria (7.40). The discrepancies between the two laboratories were in the tracheal results. The selectivity reported by Crooks and co-workers appears to reflect their high pA_2 value for 4-BIP on trachea. They calculated selectivity from a pA_2 value obtained on methacholine-contracted tracheal preparations whereas in the present study the pA_2 values from intrinsic tone preparations were used. Data were also obtained in the present study on tracheal preparations contracted by carbachol but were not used to calculate selectivity because the slopes of some of the Schild plots were not 1.0, e.g. α-methylpropranolol gave a slope of 0.82, isoprenaline as agonist. However, none of the pA_2 values for 4-BIP on trachea in the present study (intrinsic tone or carbachol-contracted) was as high as the value (8.64) quoted by Crooks *et al.* (1979). The reason for their high value is not known, particularly as they do not quote slopes of Schild plots. But one difference between their experiments and those reported in this paper is that α-adrenoceptors and uptake mechanisms were inhibited in the present study, whereas in the study of Crooks *et al.* (1979) they were not.

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