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DISSOCIATION CONSTANTS OF ISOPRENALINE AND ORCIPRENALINE AND THEIR RELATIVE EFFICACIES ON GUINEA-PIG ISOLATED ATRIA DETERMINED BY USE OF AN IRREVERSIBLE β–ADRENOCEPTOR ANTAGONIST

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1 Dissociation constants (K_A) of isoprenaline and orciprenaline were determined for the positive inotropic and chronotropic responses of guinea-pig isolated atria. Cumulative dose-response curves to the agonists were constructed before and after incubation with and washout of the irreversible β-adrenoceptor antagonist, Ro 03–7894 1-(5-chloracetylaminobenzfuran-2-yl)-2-isopropylaminoethanol).

2 After 20 min washout, the curves were displaced to the right with depression of the maxima. After 3 h washout, there was only depression of the maxima.

3 Dissociation constants were determined by plotting reciprocals of molar concentrations before Ro 03-7894 (1/A) against reciprocals of the equiactive concentrations after Ro 03-7894 (1/A'). K_A = (slope − 1)/intercept.

4 Isoprenaline had a greater affinity (K_A) than orciprenaline on both rate and tension. The affinity for rate and tension was identical for both agonists, indicating that the β-adrenoceptors were identical.

5 Isoprenaline and orciprenaline produced identical rate response maxima when compared in the same preparation but the orciprenaline tension maximum was only 92.0 ± 0.3% that of isoprenaline.

6 These dose-response curves were replotted as response against −log RA/Rt (fraction of receptors occupied) for each agonist concentration, calculated from the equation RA/Rt = (A)/(K_A + (A)). The antilogarithm of the distance along the −log RA/Rt axis gave the efficacy of orciprenaline relative to isoprenaline. It had a greater efficacy (2.24) for rate responses but a lower efficacy for tension responses (0.5).

Introduction

It has been proposed that the β-adrenoceptors mediating the positive inotropic and chronotripic responses of guinea-pig atria are identical on the basis of similar potency orders for sympathomimetic amines (Lumley & Broadley, 1977). However, there are several examples in the literature of agonists having selectivity for either the rate or force of cardiac contractions. Salbutamol and soterenol (Farmer, Kennedy, Levy & Marshall, 1970) and OPC-2009 (Yabuuchi, 1977) have been shown to exert preferential chronotropic activity in guinea-pig atria. In contrast, dopamine (Goldberg, 1972), dobutamine (Tuttle & Mills, 1975) and thyronamine (Boissier, Giudicelli, Larno & Advenier, 1973) have been found to exhibit inotropic selectivity. It is possible that some of these instances of selectivity can be attributed to the claim that in certain species there is a mixed β_1 and β_2-adrenoceptor population in the sinoatrial node but only β_1-adrenoceptors in the myocardium (Carlsson, Dahlöf, Hedberg, Persson & Tangstrand, 1977). However, in the guinea-pig atria used in the present study, no rate selectivity of the β_2-selective agonists such as salbutamol has been observed (Lumley & Broadley, 1977).

The studies mentioned above were based upon the potencies of agonists expressed as the EC_{50} values. However, the EC_{50} of an agonist may be a poor measurement of the affinity of that agonist for the receptor (Furchgott, 1966). The potency, measured as the EC_{50}, is influenced by both the affinity and the efficacy of the agonist at the receptor level (Stephenson, 1956; Besse & Furchgott, 1976). When attempting to classify receptors using a series of agonists, the individual contributions of the affinity and efficacy should be determined (Jenkinson, 1973; Triggle & Triggle, 1976). In the case of α-adrenoceptors, these

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two parameters have been calculated for a range of sympathomimetic amines by the use of an irreversible antagonist such as dibenamine (Besse & Furchgott, 1976). An irreversible antagonist of β-adrenoceptors has not been readily available. However, the characteristics of the irreversible blocking activity of Ro 03-7894 (1-(5-chloracetylaminobenzfuran-2-yl)-2-isopropylaminoethanol) on guinea-pig isolated atria have recently been described (Nicholson & Broadley, 1978). In the present study this antagonist has been used to determine the dissociation constants of isoprenaline and orciprenaline upon the positive inotropic and chronotropic responses of guinea-pig isolated atria, together with the efficacy of orciprenaline relative to isoprenaline.

Methods

Isolated atrial preparations

Guinea-pigs of either sex and weight range 300 to 500 g were killed by a blow on the head. The left and right atria were removed separately and mounted on a combined tissue holder and electrode as described previously (Broadley & Lumley, 1977). They were suspended in an organ bath (50 ml) containing Krebs-bicarbonate solution of the following composition (mM): NaCl 118.4, KCl 4.7, $CaCl_2 2H_2O$ 1.9, $NaHCO_3$ 25, $MgSO_4 7H_2O$ 1.2, glucose 11.7, $NaH_2PO_4 2H_2O$ 1.2. This was gassed with 5% CO_2 in O_2 and maintained at 38 ± 0.5°C. Each atrium was attached by means of a cotton thread to an isometric transducer (Devices, Type UF1, 28 g sensitivity range), and the tension developed was recorded on a Devices M19 polygraph. An initial diastolic tension of 0.5 to 0.8 g was applied to both atria. Inotropic responses were obtained from the left atria paced at a constant rate of 2 Hz with square wave pulses (5 ms and threshold voltage plus 50%) delivered by an SRI stimulator (Type 6053). Chronotropic responses were obtained from the spontaneously beating right atrium by means of a ratemeter (Devices, Type 2751) triggered by the tension signal.

Construction of dose-response curves

All preparations were initially allowed to stabilize for 30 min during which time several changes of bathing medium were made. A preliminary cumulative dose-response curve to the agonist (isoprenaline or orciprenaline) was constructed by approximately 3 fold increments in concentration until the maximum response was obtained. This curve served only to prime the tissue and was discarded from any calculations. After restoring the resting rate and tension to their pre-drug level, a second curve was obtained which was used for plotting purposes. The antagonist, Ro 03-7894, was then added to the bath and incubated with the tissue for 30 min before removal from the bath by one of three washout procedures. These were, three washings during 20 min or washing every 20 min for either 2 or 3 h. A final curve to the agonist was then constructed.

Small changes in sensitivity occurring during the course of the experiment were corrected for by performing control experiments for both isoprenaline and orciprenaline. These were identical in design, except for the omission of the antagonist. The mean (n = 4) total rate and total tension at each agonist concentration on the third curve of control experiments was expressed as a fraction of the values on the second curve. The range of correction factors was 0.93 to 0.97 for rate and 0.67 to 0.97 for tension. These factors were then applied to the pre-antagonist isoprenaline or orciprenaline curve of individual test experiments. The increases in rate and tension were obtained by subtracting the resting levels immediately before the dose-response curve. The resting level for the pre-antagonist curve was first corrected from the control experiments, whereas the actual resting level for the post-antagonist curve was used. These corrected increases in rate and tension were then plotted as a percentage of the maximum possible increase. This maximum response was calculated by subtracting the actual post-antagonist resting level from the pre-antagonist maximum total rate or tension. The change in resting rate to 88.3 ± 4.8% and tension to 48.4 ± 7.6% in test experiments (6.4×10^{-4}M Ro 03-7894 and 3 h washout) was not significantly different ($P > 0.05$) from changes in control experiments (97.8 ± 3.0 and 58.4 ± 12.6 respectively).

Calculation of dissociation constant (K_A)

The individual dose-response curves plotted as described above were used for calculating the dissociation constants by the method derived by Furchgott (Furchgott, 1966; Furchgott & Bursztyn, 1967; Besse & Furchgott, 1976). The equation

$$\frac{1}{(A)} = \frac{1-q}{q(K_A)} + \frac{1}{q(A')}$$

was applied to the results, where q is the fraction of active receptors remaining after irreversible blockade. The theoretical validity of this equation has been verified by Thron (1970).

The equiactive molar concentrations of isoprenaline or orciprenaline obtained before (A) and after washout (A′) of the irreversible antagonist Ro 03-7894 were obtained from individual experiments. The reciprocal values were plotted as 1/A against 1/A′ and from the calculated regression line, the dissociation constant K_A = (slope − 1/intercept.

Calculation of relative efficacy (e_r)

Orciprenaline was compared with isoprenaline in the same preparation, a curve to isoprenaline being followed by one to orciprenaline. The isoprenaline dose-response curve was corrected from control experiments as described above. The mean (n = 4) increases in rate and tension were plotted as a percentage of the corrected maximum response to isoprenaline against log molar concentration of agonist. These response values were then replotted against the negative logarithm of the fraction of receptors occupied (RA/Rt) by each concentration (A). This value was obtained for each molar concentration (A) by substitution into the equation RA/Rt = (A)/(K_A + (A)). The K_A values used were those derived by the use of the irreversible antagonist. The efficacy (e_r) of orciprenaline relative to isoprenaline was the antilogarithm of the distance along the RA/Rt axis between the orciprenaline curve and the isoprenaline reference (Furchgott & Bursztyn, 1967; Besse & Furchgott, 1976).

Drugs

The antagonist Ro 03-7894 (1-(5-chloracetylamino-benzfuran-2-yl)-2-isopropylaminoethanol) as the base was synthesized in the chemistry laboratories of Roche Products Ltd., Welwyn Garden City. (−)-Isoprenaline bitartrate dihydrate (Ward Blenkinsop) and (±)-orciprenaline sulphate (Boehringer Ingelheim) were kindly supplied as gifts. All stock solutions were freshly prepared in distilled water. Ascorbic acid was added to the isoprenaline and orciprenaline solutions (1 μg/ml) and the Ro 03-7894 (1 mg/ml) to aid solution.

Results

The positive inotropic and chronotropic responses to isoprenaline and orciprenaline were antagonized by Ro 03-7894 at a concentration of 3.2×10^{-4} or 6.4×10^{-4}M. The antagonist was washed from the bath for either 20 min or 3 h before constructing the final dose-response curve. When the 6.4×10^{-4}M concentration and the 20 min washout time were used, the rate and tension dose-response curves to isoprenaline were displaced to the right with a depression of the maximum to 75.9 ± 7.8 and 81.1 ± 10.2% respectively (Figure 1a). These maxima were not significantly different (P >0.05). The depressions of the orciprenaline rate and tension maxima to 47.8 ± 7.9 and 53.8 ± 6.3% were significantly (P <0.05) greater (Figure 1b). When the washout time was extended to 3 h the shift of the dose-response curve to the right was considerably reduced but the maximum response was depressed (Figure 2). In the case of isoprenaline this depression was significantly greater (P <0.05) than for the 20 min washout time, the values for rate and tension being 52.3 ± 7.8 and 45.6 ± 3.2% respectively.

The dissociation constants of isoprenaline and orciprenaline were then calculated from the data obtained with each concentration and washout time of Ro 03-7894. The equiactive molar concentrations obtained from the mean (n = 4) dose-response curves for isoprenaline and orciprenaline constructed before the 6.4×10^{-4}M concentration of Ro 03-7894 and after a 3 h washout are shown in Figure 3. Plotting the reciprocals of the concentration before Ro 03-7894 (1/A) against the reciprocal of the value after the antagonist (1/A′) yielded straight lines for the rate (Figure 3a and b) and tension (Figure 3c and d) responses.

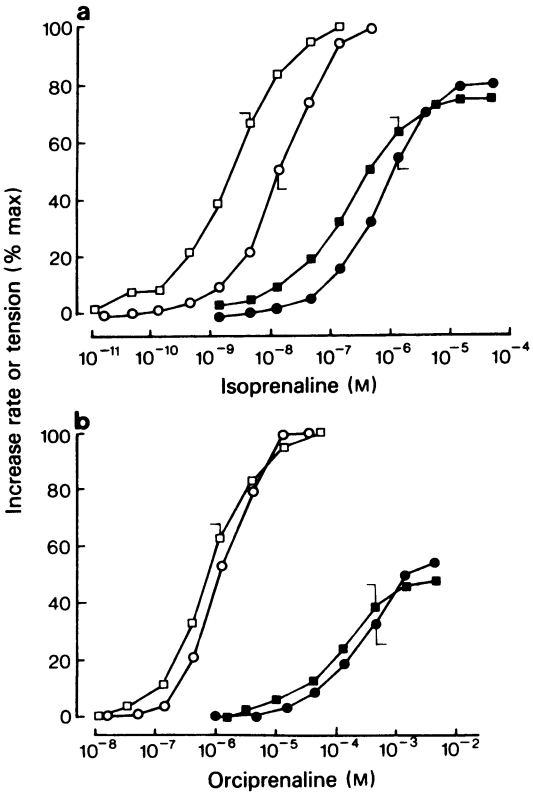

Figure 1 Effect of Ro 03-7894 on dose-response curves to isoprenaline and orciprenaline of guinea-pig isolated atria. Mean (n = 4) dose-response curves for the positive inotropic (○) and chronotropic (□) responses to isoprenaline (a) and orciprenaline (b) were constructed before (open symbols) and after Ro 03-7894 (solid symbols). The tissue was incubated with Ro 03-7894 (6.4×10^{-4}M) for 30 min and the bath washed 3 times during 20 min before constructing the final curve. Vertical bars on one point on each curve represent the s.e.mean.

Dissociation constants (K_A) were determined from individual experiments and the mean values (± s.e. mean) then calculated. The values obtained with each experimental design are shown in Table 1. Under all conditions, for both rate and tension, isoprenaline had greater affinity (dissociation constant) than orciprenaline. It is apparent that the K_A values for isoprenaline and orciprenaline were not constant between the different washout times. As the washout time was increased, the K_A values were significantly ($P < 0.05$) reduced whether isoprenaline- or orciprenaline-induced rate or tension responses were used. The difference appears to occur between 20 min and 2 h since there was no further reduction of the K_A values between 2 and 3 h obtained with isoprenaline and the 6.4×10^{-4}M concentration of Ro 03-7894. The K_A values for isoprenaline and orciprenaline on both rate and tension did not vary with the concentration of Ro 03-7894 used, providing the same washout time was employed.

When rate and tension were compared, there was some difference in K_A value when the 20 min washout time was used. However, at the longer washout time the values for rate and tension were much closer and did not differ significantly ($P > 0.05$) for either isoprenaline or orciprenaline.

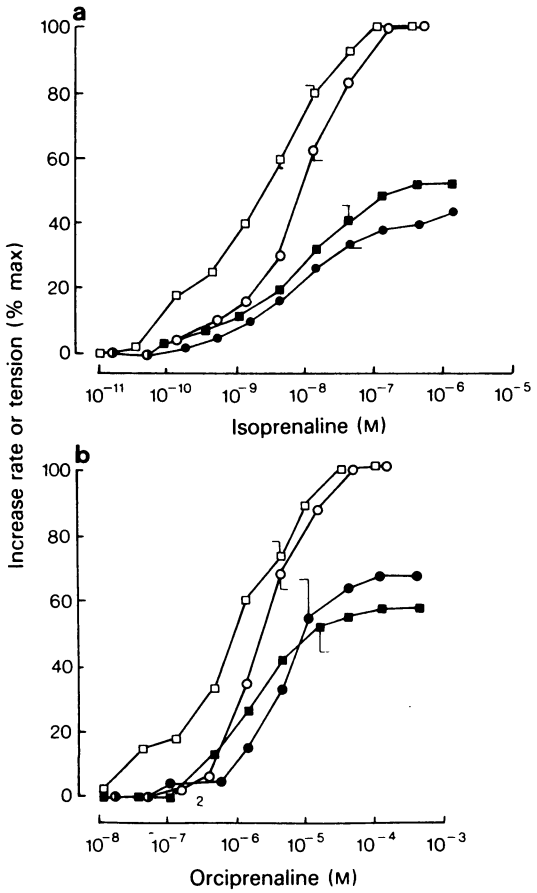

Figure 2 Effect of Ro 03-7894 on dose-response curves to isoprenaline and orciprenaline of guinea-pig isolated atria. Mean ($n = 4$) dose-response curves for the positive inotropic (○) and chronotropic (□) responses to isoprenaline (a) and orciprenaline (b) were constructed before (open symbols) and after Ro 03-7894 (solid symbols). The tissue was incubated with Ro 03-7894 (6.4×10^{-4}M) for 30 min and the bath washed out every 20 min for 3 h before constructing the final curve. Vertical bars on one point on each curve represent the s.e.mean.

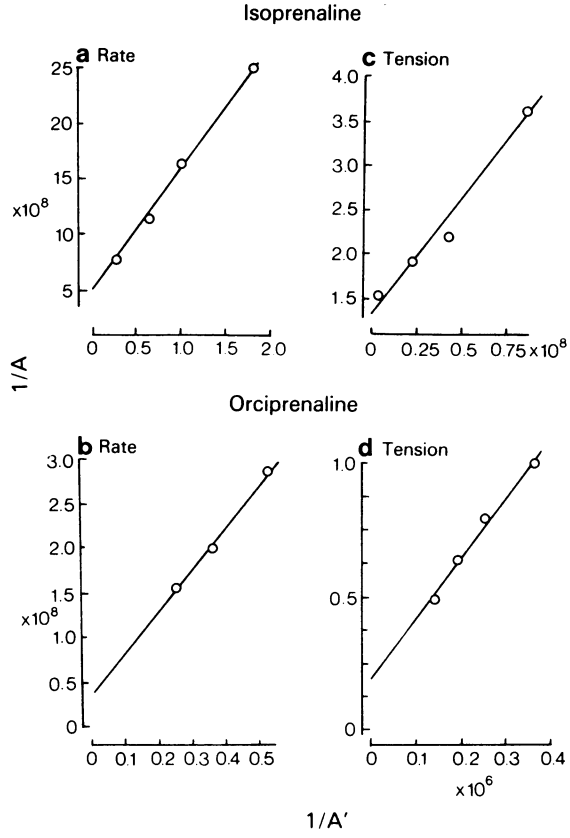

Figure 3 Double reciprocal plots of equiactive concentrations of isoprenaline and orciprenaline before and after Ro 03-7894. Reciprocals of the molar concentration before antagonist (1/A) are plotted against the reciprocals of equiactive molar concentrations after Ro 03-7894 (1/A'). These values were obtained from the mean ($n = 4$) dose-response curves for the positive chronotropic (a and b) and inotropic (c and d) responses of guinea-pig isolated atria to isoprenaline (a and c) and orciprenaline (b and d), using the 6.4×10^{-4}M concentration of Ro 03-7894 and 3 h washout shown in Figure 2. Dissociation constants (K_A) were calculated as (slope −1)/intercept.

The fraction of receptors unoccupied by the antagonist (q) was calculated for each experimental design, the mean (± s.e. mean) of the values obtained from individual experiments being shown in Table 1. Four points regarding these q values could be identified. (1) There was no significant difference ($P > 0.05$) between q values for the rate and tension, except at the 3.2×10^{-4}M concentration of Ro 03-7894 with a 20 min washout. Here, the maximum rate response was depressed significantly ($P < 0.05$) more than the tension response by Ro 03-7894. (2) There was some discrepancy between the q values obtained with isoprenaline and orciprenaline, although on theoretical grounds they should be identical and independent of the agonist used. (3) As the concentration of Ro 03-7894 was raised, the fraction of receptors unoccupied (q) was consistently reduced. (4) The effect of extending the washout time was apparently to increase the fraction of unoccupied receptors (q). Therefore washout appeared to remove the antagonist from the receptors.

Finally, the efficacy of orciprenaline (e_r) relative to isoprenaline was determined. The two agonists were therefore examined in the same preparation. A cumulative dose-response curve to isoprenaline (after the preliminary curve) was constructed, followed by one to orciprenaline. The mean ($n = 4$) rate (Figure 4a) and tension (Figure 4b) increases were plotted against log molar concentration of agonist. The maximum rate response to orciprenaline was not significantly different ($P > 0.05$) from that to isoprenaline, but the maximum tension response (92.0 ± 0.3%) was significantly ($P < 0.05$) less than isoprenaline. These dose-response curves were then replotted as the response against log RA/Rt for both rate (Figure 4c) and tension (Figure 4d). The value of RA/Rt for each concentration of agonist (A) was calculated by substitution in the equation $RA/Rt = (A)/(K_A + (A))$. The K_A values for isoprenaline and orciprenaline used were those obtained with the 6.4×10^{-4}M concentration of Ro 03-7894 and the 3 h washout. These were selected because prolonged washout had little further effect upon the K_A value between 2 and 3 h and it was probably stable at 3 h. The efficacy of orciprenaline obtained from these graphs was greater than isoprenaline for the rate responses (2.24), whereas for tension responses (0.50) it was less than isoprenaline.

Table 1 Mean dissociation constants (K_A) (± s.e.mean $n = 4$) for the positive chronotropic and inotropic responses of guinea-pig isolated atria of isoprenaline and orciprenaline using an irreversible β-adrenoceptor antagonist, Ro 03-7894 and the proportion of receptors left unoccupied (q) by that antagonist.

Concentration of Ro 03-7894	*Washout time* (min)	*Isoprenaline*				*Orciprenaline*			
		Rate		*Tension*		*Rate*		*Tension*	
		$K_A \times 10^{-4}$M	q	$K_A \times 10^{-4}$M	q	$K_A \times 10^{-6}$M	q	$K_A \times 10^{-6}$M	q
3.2×10^{-4}M	20	57.8 ± 11.2	0.012 ± 0.008	199.4 ± 82.1	0.056 ± 0.031	323.0 ± 28.1	0.01 ± 0.008	571.6 ± 108.1	0.056 ± 0.038
	180	3.8 ± 1.8	0.56 ± 0.32	2.4 ± 2.3	0.63 ± 0.52				
6.4×10^{-4}M	20	64.0 ± 11.0	0.007 ± 0.003	169.9 ± 70.3	0.012 ± 0.10	289.1 ± 98.3	0.003 ± 0.001	432.0 ± 62.1	0.005 ± 0.002
	120	1.7 ± 0.91	0.21 ± 0.18	2.2 ± 1.4	0.20 ± 0.05				
	180	2.9 ± 1.3	0.17 ± 0.09	2.3 ± 0.7	0.17 ± 0.08	10.0 ± 2.2	0.65 ± 0.42	2.4 ± 1.3	0.54 ± 0.32

*Bracketed values only are significantly different ($P < 0.05$) as determined by Students' t test.

Discussion

The dissociation constants (K_A) of isoprenaline and orciprenaline were determined for the β-adrenoceptors mediating the positive inotropic and chronotropic responses of guinea-pig isolated atria. The method used the antagonist Ro 03-7894 which has been shown previously to behave in an irreversible manner and not to depress the tissue by a non-specific

effect independent of β-adrenoceptors, as measured by its failure to affect the responses to calcium (Nicholson & Broadley, 1978). The K_A values were determined with various conditions of washout and concentration. The K_A value of an agonist on any parameter is a theoretical constant of the drug-receptor interaction (see Furchgott, 1972) and should therefore be independent of variables such as these. This however was clearly not the case. Although it remained approximately constant between the concentration of Ro 03-7894 employed, there was a marked fall in the K_A values after longer washout times. These discrepancies between K_A values were accompanied, at the various washout times, by differences in the shifts of the dose-response curves induced by Ro 03-7894. After the prolonged washout time there was apparently little rightwards shift of the dose-response curves but only a depression of the maximum responses by the antagonist. This characterizes the antagonism as irreversible and non-equilibrium (Nickerson, 1957). It is possible that, as suggested previously (Nicholson & Broadley, 1978), incomplete washout of the antagonist that is not irreversibly bound to the β-adrenoceptors, results in some competitive antagonism. Identical behaviour has been reported for the irreversible β-adrenoceptor antagonist FM 24 (Lucas, Homburger, Dolphin & Bockaert, 1979). A simultaneous competitive antagonism would introduce a complicating factor to the equations derived for determining the K_A values

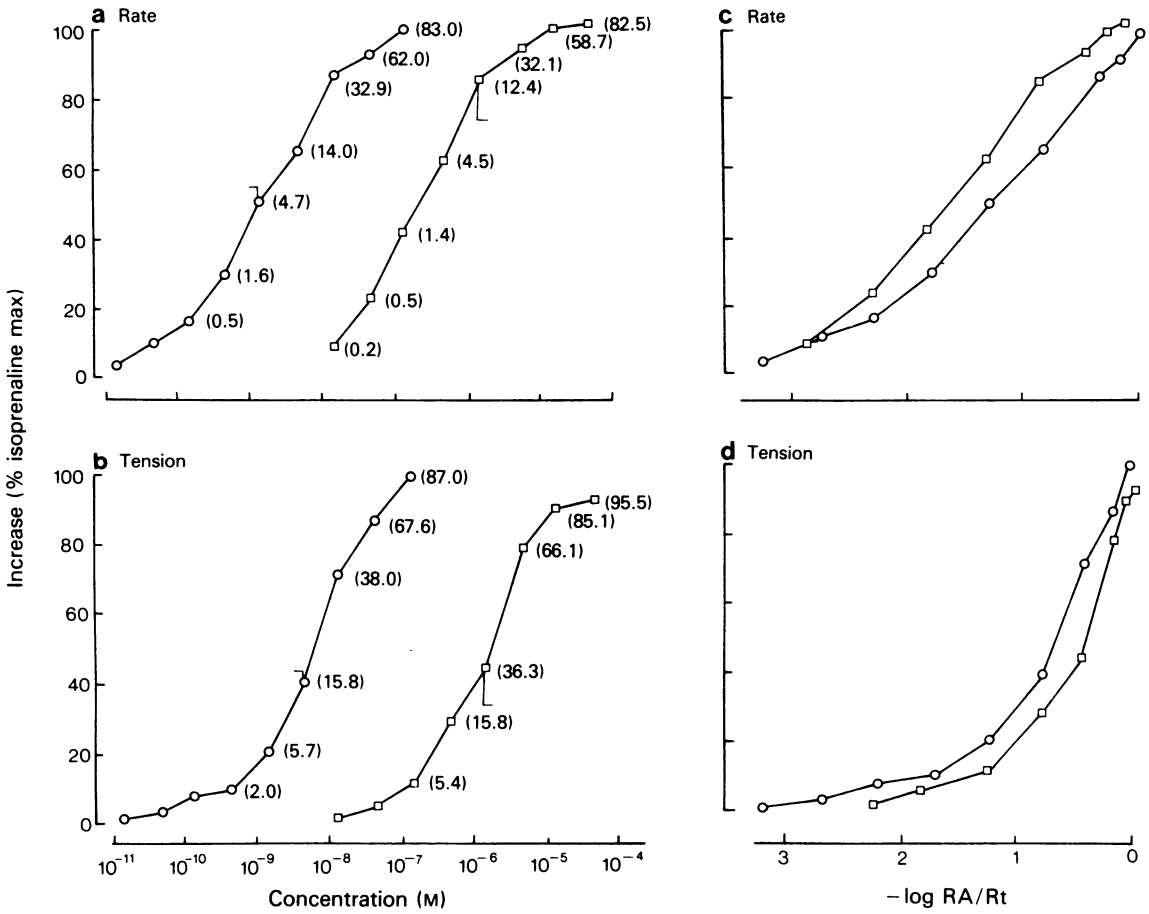

Figure 4 Comparisons of isoprenaline and orciprenaline in the same guinea-pig isolated atria. In (a) and (b), the mean (n = 4) positive chronotropic and inotropic responses to isoprenaline (○) and orciprenaline (□) were plotted against molar concentration. In (c) and (d), these responses were replotted against the −log RA/Rt value for each concentration, calculated from the equation RA/Rt = (A)/(K_A + (A)). The K_A values used were those obtained with 6.4 × 10^{-4}M Ro 03-7894 and the 3 h washout. The antilog of the distance along the abcissa scale between these isoprenaline and orciprenaline curves yielded the relative efficacy (e_r). Values in parentheses are the percentage receptors occupied by the agonist concentration calculated from the same equation.

and would lead to an overestimate of the K_A value (Besse & Furchgott, 1976). The more accurate assessment of K_A value was therefore considered to be that derived from the longest washout time employed.

Under these conditions the K_A values for rate and tension responses were virtually identical for both isoprenaline and orciprenaline. The small difference for orciprenaline was not significant. This agrees with results obtained by ourselves (Broadley & Nicholson, 1978; 1979) and others (Buckner, Torphy & Costa, 1978) in which functional antagonism was used to determine the dissociation constants. This finding is also consistent with the observations that the affinity values (pA_2) of competitive antagonists are identical for the positive inotropic and positive chronotropic responses (Blinks, 1967; Bristow & Green, 1970; Horii, Kawada, Takeda & Imai, 1974; Lumley & Broadley, 1975; 1977). This provides further confirmatory evidence that the β-adrenoceptors subserving these two responses are identical. This also supports the contention that the β-adrenoceptor population is homogeneous as proposed by O'Donnell & Wanstall (1979), at least for the rate responses of guinea-pig atria. Radioligand binding studies however throw some doubt upon this, suggesting a heterogeneous population in right atria (Hedberg, Minneman & Molinoff, 1979).

Isoprenaline and orciprenaline, like most sympathomimetic amines (Lumley & Broadley, 1977), were rate selective at the 38°C bath temperature used, orciprenaline exhibiting a lower maximum response on tension than isoprenaline. This selectivity, from the current evidence, could not be explained by affinity differences.

Orciprenaline had a lower affinity than isoprenaline on both rate and tension by factors of 30 and 100 fold respectively. These differences and the K_A values themselves compare favourably with the values obtained by functional antagonism (Broadley & Nicholson, 1979). Differences in K_A value and potency of agonists can be modified by factors that affect their concentration in the vicinity of the receptor. For example, the order of potency of catecholamines in guinea-pig isolated atria is altered when neuronal uptake is inhibited by cocaine (Furchgott, 1967). In addition, the K_A values may be overestimated if extraneuronal uptake is not inhibited (Besse, 1975). However interference from these sources could be discounted since isoprenaline and therefore its structural isomer, orciprenaline, are not subject to neuronal uptake (Burgen & Iversen, 1965). Similarly inhibition of catechol *O*-methyltransferase (COMT) by tropolone and of extraneuronal uptake by metanephrine have negligible enhancing activity upon the responses of guinea-pig isolated atria to isoprenaline and orciprenaline (Broadley & Duncan, 1977; Duncan, 1978).

The data from which the K_A values were calculated also provided an estimate of the fraction of receptors not occupied (q) by the irreversible antagonist. Predictably, this fraction fell as the concentration of Ro 03-7894 was increased. As with the K_A values, the q values calculated with the shorter washout times are probably not an accurate indication of the true number of free receptors.

The q values should be independent of the agonist employed yet small differences between isoprenaline and orciprenaline were obtained. A possible explanation is that these agonists were not examined in the same preparation. It is reasonable to expect that the number of receptors will vary between preparations and therefore the proportion of receptors unoccupied by a concentration of Ro 03-7894 (q) will also vary to some extent. This might also explain the discrepancies in the depression of maximum responses that occurred between isoprenaline and orciprenaline.

A comparison of the positive inotropic and chronotropic responses showed that neither rate nor tension maxima were consistently depressed more when isoprenaline or orciprenaliane was the agonist. A difference might have been anticipated however, since orciprenaline failed to produce the same maximum tension response as isoprenaline and could be classed as a partial agonist. As such it would be expected to occupy a greater proportion of the receptors than isoprenaline to produce its maximum response (Stephenson, 1956). In fact, calculation of the receptor occupancy (RA/Rt) showed that at the tension maxima, isoprenaline occupied 87.0% whereas orciprenaline occupied 95.5% of the receptors. Therefore, one could conclude that there would be very little, if any, receptor reserve at the maximum response for either agonist. A similar conclusion was reached by Besse & Furchgott (1976) for full agonists at α-adrenoceptors in rabbit aorta.

Comparison between rate and tension also showed that for half maximal responses, isoprenaline occupied approximately 5% of the receptors for rate and 20% for tension responses. The rate selectivity of isoprenaline may therefore arise because it must occupy a greater proportion of receptors for tension than for rate responses. This contrasts with the observation of Bucker *et al.* (1967) who calculated the same occupancy for each response in rat atria.

Athough orciprenaline had a lower affinity than isoprenaline on both rate and tension, its efficacy was over twice that of isoprenaline on rate responses. This is dependent upon the K_A value, which may show some between-tissue variation, however, the authenticity of this observation is supported by an identical finding in an earlier study. In this, the efficacy was derived from K_A values determined by the alternative method of functional antagonism (Broadley & Nicholson, 1978; 1979). It is also substantiated by the observation of O'Donnell & Wanstall (1977) who showed that the catecholamine derivative, Me 454,

could produce a greater maximum rate response than isoprenaline in the presence of the functional antagonist carbachol. We have obtained a similar result with orciprenaline in the presence of carbachol (Broadley & Nicholson, 1979) and both observations could be attributed to a greater efficacy. With regard to the tension responses however, orciprenaline had an efficacy only half that of isoprenaline. This might explain why it failed to achieve the same tension maximum as isoprenaline, yet it was able to produce the same rate maximum as isoprenaline. The efficacy difference between isoprenaline and orciprenaline may also account for the fact that the maximum responses to orciprenaline were not depressed by Ro 03-7894 as much as expected. Receptor occlusion alone does not control the degree of maximum response depression, agonists with dissimilar efficacies will be affected differently.

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