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Evidence from agonist and antagonist studies to suggest that the β_1-adrenoceptors subserving the positive inotropic and chronotropic responses of the heart do not belong to two separate subgroups

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The positive inotropic and chronotropic responses of guinea-pig isolated left and right atria to 17 sympathomimetic amines were examined under conditions selected to control the pharmacological environment. Each agonist was compared with (−)-isoprenaline as the reference by constructing dose-response curves. Equiactive molar concentration ratios relative to (−)-isoprenaline were calculated at the EC50 for rate and tension responses. Statistical analysis revealed that (−)-noradrenaline and (±)-α-methylnoradrenaline were tension selective whereas (±)-α-ethylisoprenaline and *N*-methyldopamine were rate selective relative to (−)-isoprenaline. However, no structural trends emerged. The rank order of potency varied slightly between rate and tension, but an analysis of the regression and correlation coefficients indicated respectively that the equiactive molar concentration ratios and the rank orders could be considered identical on rate and tension. Antagonism of the (−)-isoprenaline-induced rate and tension responses by acebutolol, atenolol, practolol and propranolol was assessed from the pA_2 values which were almost identical for both parameters with each antagonist. It is concluded that the β_1-adrenoceptors mediating the positive inotropic and chronotropic responses do not warrant subdivision into two separate groups.

The β-adrenoceptors mediating the positive inotropic and chronotropic effects of sympathomimetic amines on the heart have been subclassified as the β_1-type (Lands, Arnold & others, 1967). However there are reports of agonists exerting selective effects upon either the rate or the force of contraction. For example, soterenol and salbutamol have been shown to have selective chronotropic activity on guinea-pig isolated atria (Farmer, Kennedy & others, 1970). In contrast dopamine has been shown to preferentially stimulate the force of cardiac contractions *in vivo* (Goldberg, 1972). This observation resulted in the synthesis of dobutamine, an analogue of dopamine which also has selective inotropic activity (Tuttle & Mills, 1975; Lumley, Broadley & Levy, 1977) and has been used clinically in the treatment of left ventricular dysfunction (Jewitt, Birkhead & others, 1974). Similarly thyronamine, the decarboxylation product of thyronine, preferentially stimulates the force of contraction, a response that is antagonized by pindolol and therefore mediated via β-adrenoceptors (Boissier, Giudicelli & others, 1973). These reports suggest that the β-adrenoceptors for the rate and force responses of the heart may differ. Dreyer & Offermeier (1975) have examined this possibility by determining the relative activities of a range of sympathomimetic amines upon the rate and force of contraction of guinea-pig isolated atria and concluded that the adrenoceptors mediating these responses were different. The same conclusion was reached by Giudicelli (1975) using a more limited range of agonists. Both groups of workers pointed out limitations of their results since other factors such as indirect sympathomimetic activity were not prevented by suitable pretreatment, although Dreyer & Offermeier (1975) claimed that pretreatment with cocaine did not alter their results and conclusions.

The evidence from the literature reviewed above would therefore appear to support the concept of two different β_1-adrenoceptor types mediating rate and force responses. However, in view of possible limitations in the work of Dreyer & Offermeier (1975) and Giudicelli (1975) we have examined a range of sympathomimetic amines under conditions designed to control the pharmacological environment. These were aimed at excluding neuronal and extraneuronal uptake, indirect activity and possible stimulation of myocardial α-adrenoceptors (Govier, 1968), so that any differences arising between the rate and force could hopefully be attributed only to

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respective β_1-adrenoceptors themselves (Furchgott, 1972).

Although studies with agonists were originally used for the classification of adrenoceptors (Ahlquist, 1948) and are still favoured by some workers (Arnold & McAuliff, 1971), they are open to certain limitations (Furchgott, 1972). A more reliable quantitative assessment of any receptor differences may be obtained from calculations of affinity constants of competitive antagonists. Therefore to complement our studies with agonists, the blockade by several β-adrenoceptor antagonists of the isoprenaline-induced rate and tension responses has been compared. By these two approaches it was hoped to determine whether the β_1-adrenoceptors responsible for rate and tension belong to two separate subgroups. Such a finding would have potential therapeutic application in the development of selective inotropic agents for the treatment of myocardial failure.

MATERIALS AND METHODS

Guinea-pigs of either sex, 400–600 g, were killed by a blow on the head and exsanguinated under running water. The thorax was rapidly opened and left and right atria removed separately as described previously (Broadley & Lumley, 1977). The atria were mounted on a combined tissue holder and electrode and suspended in a 50 ml organ bath containing Krebs-bicarbonate solution (composition in 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) gassed with 5% CO_2 in oxygen and maintained at 38 ± 0·5°. Each atrium was attached by cottons to transducers (Devices, Type UF 1, 57 g sensitivity range) for recording isometric tension on a Devices M19 polygraph. Initial resting diastolic tensions of 1 and 0·5 g were applied to the left and right atria respectively. Positive inotropic responses were obtained from the left atria paced at a constant rate of 2·5 Hz with square wave pulses (5 ms and threshold voltage + 50%) delivered by an SRI stimulator (Type 6053). Chronotropic responses were recorded by means of a ratemeter (Devices, Type 2751) triggered by the tension signal from the spontaneous right atrium.

All preparations were allowed to stabilize for 20 min during which several changes of the bathing medium were made. An initial cumulative dose-response curve to (−)-isoprenaline was constructed using 10-fold increments in concentration until the maximum response was obtained. After the tissue had been washed and the rate and tension had returned to their pre-drug levels, a second cumulative dose-response curve to (−)-isoprenaline was obtained using 3-fold increments of concentration.

Agonist studies

Control of the pharmacological environment was achieved by using atria from guinea-pigs pretreated with reserpine, a dose of 0·5 mg kg^{-1} (i.p.) 24 h before use of the animals having been found satisfactory to prevent indirect sympathomimetic activity (Broadley & Lumley, 1977). Also, to irreversibly inhibit neuronal and extraneuronal uptake, and therefore degradation by COMT and stimulation of α-adrenoceptors, the atria were incubated with phenoxybenzamine ($1{\cdot}64 \times 10^{-5}$ M) for 20 min following the initial stabilization period (Furchgott, 1972). After three changes of fresh Krebs-bicarbonate solution during a further 20–30 min interval, the first and second cumulative dose-response curves were constructed. After washout, a third cumulative dose-response curve to the agonist under study was obtained. The responses were measured as the increases in rate and tension above the pre-drug level at each concentration. It has been the experience of ourselves and others (Levy—personal communication) that the slopes of the first and second dose-response curves to isoprenaline differ, whereas the second and third curves are virtually the same. Application of this slope difference to the test agonist was thus avoided by discarding the initial curve to (−)-isoprenaline and making comparisons of the test agonist with the second (−)-isoprenaline curve. Control experiments were performed, in which the tissues were exposed to a third (−)-isoprenaline curve in place of the test agonist. Only small changes in response size occurred between the second and third curves, but the means (n = 4) of any differences at each concentration were expressed as percentages and these factors applied to the individual (−)-isoprenaline reference curves of test experiments.

Antagonist studies

pA_2 values for the antagonism of (−)-isoprenaline-induced rate and tension responses (of untreated preparations) were calculated by the method of Arunlakshana & Schild (1959). Following the first two dose-response curves to (−)-isoprenaline, the antagonist was incubated with the tissue for 45 min before a third curve to (−)-isoprenaline was constructed in its presence. The increased rate and increased tension responses were expressed as a percentage of the maximum response and the dose-ratio

(DR) between the second and third curves calculated at the 50% level. Any small changes in sensitivity of the preparation were adjusted for by performing control experiments that were exposed to three dose-response curves to (−)-isoprenaline without the intervention of the antagonist. Any shift between the mean (n = 4) second and third control curves was expressed as a dose-ratio. The individual dose-ratios due to the antagonist were corrected for these sensitivity changes by either multiplying or dividing by the correction dose-ratio. The mean corrected dose-ratios (n = 3, except for atenolol where n = 2) for three concentrations of each antagonist were calculated and log (DR-1) plotted against molar concentration (on a log scale). From the calculated regression lines, the slope and pA_2 values were obtained, with their 95% confidence limits.

The following drugs were used: (−)-adrenaline base (Hopkin & Williams), dopamine hydrochloride, (−)-phenylephrine hydrochloride (Sigma), (−)-noradrenaline bitartrate, (±)-synephrine tartrate (Koch-Light) and reserpine (BDH). In addition, the following were supplied as gifts: (±)-acebutolol hydrochloride (May & Baker); (±)-atenolol base, (±)-practolol base, (±)-propranolol base (ICI); dobutamine hydrochloride (Eli Lilly); (−)-erythro-α-ethylnoradrenaline (+)-bitartrate monohydrate, (±)-erythro-α-methyladrenaline hydrochloride, (±)-erythro-α-methylisoprenaline hydrochloride (Dr A. Arnold, Sterling-Winthrop); (±)-erythro-α-ethylisoprenaline, (±)-α-methyldopamine hydrochloride, *N*-methyldopamine hydrochloride (Dr R. Newton, Allen & Hanburys); (±)-erythro-α-methylnoradrenaline (Hoechst A.G.); (−)-isoprenaline bitartrate dihydrate (Ward Blenkinsop); (−)-orciprenaline base (Boehringer Ingelheim); phenoxybenzamine hydrochloride (Smith, Kline & French); (±)-salbutamol base (Allen & Hanburys); (±)-soterenol hydrochloride (BDH).

RESULTS

With (−)-isoprenaline as the reference in each preparation, the increased rate and increased tension responses to 17 sympathomimetic amines were recorded. They were expressed as a percentage of the corrected (−)-isoprenaline maximum response corresponding to each agonist and the mean curves are plotted in convenient groups in Figs 1, 2 and 3.

(−)-Isoprenaline was the most active agonist examined on both rate and tension, thus justifying its selection as the reference. The relative activity of each compound was then compared quantitatively with that of (−)-isoprenaline on rate and on tension

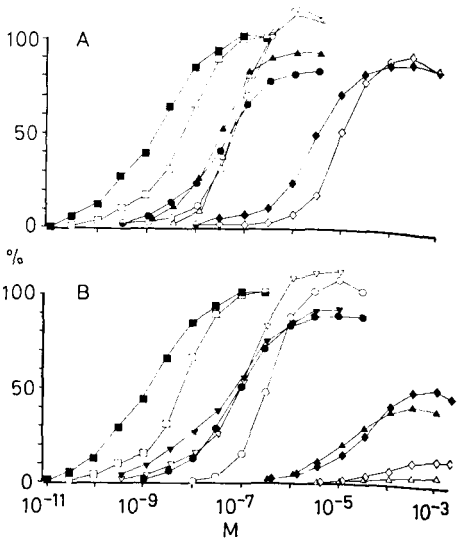

FIG. 1. Mean (n = at least 4) cumulative dose-response curves for the positive inotropic (open symbols) and positive chronotropic (closed symbols) responses of guinea-pig isolated atria to sympathomimetic amines. Each agonist was compared with (−)-isoprenaline, the responses to which were corrected for sensitivity changes from control experiments. Responses to the agonist and (−)-isoprenaline are expressed as a percentage of the (−)-isoprenaline maximum response. The (−)-isoprenaline curves shown in A and B are mean curves from the corresponding group of agonists. The preparations were taken from animals pretreated with reserpine (0·5 mg kg^{-1}, i.p. 24 h before use) and incubated with phenoxybenzamine ($1{\cdot}64 \times 10^{-5}$M) for 20 min which was removed from the bath 30 min before constructing the first dose-response curve. A. □, (−)-isoprenaline; △, (−)-noradrenaline; ○, (±)-erythro-α-methylnoradrenaline; ◇, (−)-erythro-α-ethylnoradrenaline. B. □, (−)-isoprenaline; ▽, (−)-adrenaline; ○, (±)-erythro-α-methyladrenaline; △, (−)-phenylephrine; ◇, (±)-synephrine.

as the equiactive molar concentration ratios. These were calculated by dividing the molar EC50 value for each agonist by that for (−)-isoprenaline in individual experiments. The mean values along with their 95% confidence limits are shown in Table 1. The compounds are arranged in rank order of potency on rate and tension according to their activity relative to isoprenaline. This order was similar on each parameter.

By inspection of Figs 1, 2 and 3, (−)-isoprenaline was clearly rate-selective and this was apparent with all other agonists to a greater or lesser extent. To determine whether any compounds had rate or tension selectivity significantly different from that exhibited by the (−)-isoprenaline reference, the individual equiactive molar concentration ratios for rate and tension were subjected to Student's *t*-test. For most of the compounds these ratios did not

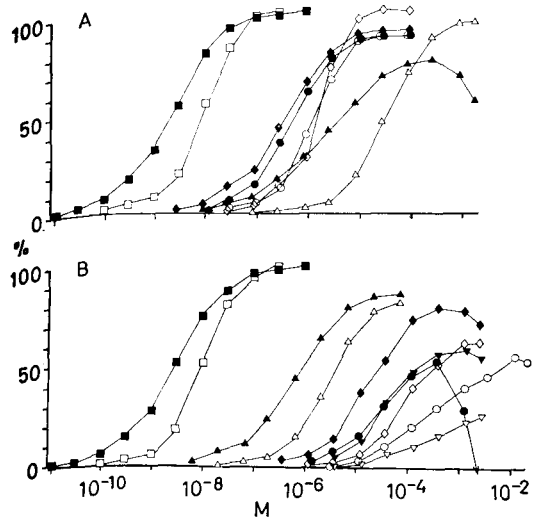

FIG. 2. Mean cumulative dose-response curves for the positive inotropic (open symbols) and positive chronotropic (closed symbols) responses of guinea-pig isolated atria to sympathomimetic amines obtained as in Fig. 1. A. □, (—)-isoprenaline; ◇, (±)-erythro-α-methylisoprenaline; △, (±)-erythro-α-ethylisoprenaline; ○, (—)-orciprenaline. B. □, (—)-isoprenaline; △, dobutamine, ▽, dopamine; ◇, *N*-methyldopamine; ○, (±)-α-methyldopamine.

differ significantly ($P > 0.05$) as shown in Table 1. However, in four cases the ratios relative to (−)-isoprenaline significantly ($P < 0.05$) differed between rate and tension. Tension selectivity was revealed by (−)-noradrenaline and (±)-α-methylnoradrenaline; at the other extreme were (±)-α-ethylisoprenaline and *N*-methyldopamine exhibiting rate selectivity.

Many of the compounds examined failed to attain the same maximum response as (−)-isoprenaline and as such could be classed as partial agonists (Figs 1, 2 and 3). This was represented in Table 1 as the relative intrinsic activity (RIA) calculated from the maximum response as a fraction of the isoprenaline maximum. Small changes in maxima were encountered on repeating dose-response curves even in control experiments. However, where this difference in the maxima (measured as the total parameter) between the test agonist and (−)-isoprenaline did not differ significantly ($P > 0.05$) from that obtained in the (−)-isoprenaline control experiments, the compound was classed as a full agonist and given a RIA of unity.

To determine whether the small, but significant, degree of selectivity found with four compounds was indicative of a receptor differentiation, the equiactive molar concentration ratios were subjected to a regression analysis. The individual values were converted to natural logarithms and the mean values

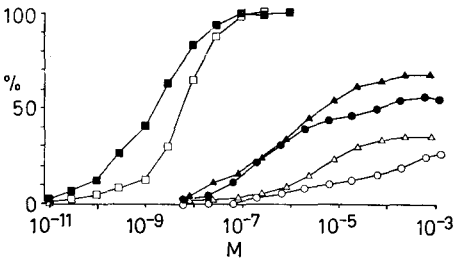

FIG. 3. Mean cumulative dose-response curves for the positive inotropic (open symbols) and positive chronotropic (closed symbols) responses of guinea-pig isolated atria to (—)-isoprenaline (□), (±)-salbutamol (△) and soterenol (○) obtained as in Fig. 1.

for increase tension ($\log_e x$) plotted against the mean value for increase rate responses ($\log_e y$). These values were also treated in the reverse manner, that is $\log_e y$ plotted against $\log_e x$. The regression ($\log_e x$ on $\log_e y$) and correlation coefficients for the analysis of all the agonists were 1·08 and 0·96 respectively (Table 2).

In view of the limitations of using partial agonists for calculations of EC50 values (Furchgott, 1972), a regression analysis was also applied to the sympathomimetic amines excluding the *weak* partial agonists. The graphical presentation of the regression analysis in Fig. 4 shows the two regression lines to be virtually superimposable; a situation that can only arise if the

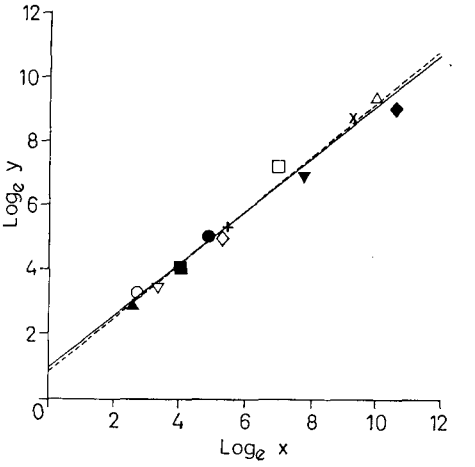

FIG. 4. Result of the regression analysis of equiactive molar concentration ratios of sympathomimetic amines classed as full agonists relative to (—)-isoprenaline on tension ($\log_e x$) plotted against the corresponding value on rate ($\log_e y$). The dashed line is that calculated when the axes were reversed. ▲, (±)-erythro-α-methylnoradrenaline; ○, (—)-noradrenaline; ▽, (—)-adrenaline; ■, (±)-erythro-α-methyladrenaline; ●, (—)-orciprenaline; ◇, dobutamine, +, (±)-erythro-α-methylisoprenaline; □, (—)-erythro-α-ethylnoradrenaline; ▼, (±)-erythro-α-ethylisoprenaline; ×, *N*-methyldopamine; △, dopamine; ◆, (±)-α-methyldopamine.

Table 1. *Positive inotropic and chronotropic activities of 17 sympathometric amines on guinea-pig isolated atria.* Results expressed as mean equiactive concentration ratios relative to (−)-isoprenaline on increased tension and rate with their 95% confidence limits in brackets. Agonists are arranged in rank order of activity on each parameter. Relative intrinsic activities were calculated as described in the text.

Increased rate			Increased tension		
Agonists (Rank order)	Equiactive conc. ratio	RIA	Agonists (Rank order)	Equiactive conc. ratio	RIA
(—)-Isoprenaline	1	1	(—)-Isoprenaline	1	1
(±)-α-Methylnoradrenaline	17·9 (13·4–24)	1	(±)-α-Methyl noradrenaline	12·6 (9·2–17·2)†	1
(—)-Noradrenaline	25·9 (17·6–38·2)	1	(—)-Noradrenaline	14·5 (11·3–18·8)*	1
(—)-Adrenaline	30·9 (16·4–58·3)	1	(—)-Adrenaline	22·4 (8·9–56·3)	1
(±)-α-Methyladrenaline	56·9 (41–78·6)	1	(±)-α-Methyl adrenaline	58·1 (40·5–83·4)	1
Dobutamine	146 (116–183)	0·85	(—)-Orciprenaline	127 (67·6–238)	1
(—)-Orciprenaline	153 (83–280)	0·88	Dobutamine	195 (135–280)	0·88
(±)-α-Methylisoprenaline	200 (164–243)	1	(±)-α-Methyl-isoprenaline	227 (143–360)	1
(±)-Soterenol	322 (73–1413)	0·56	(±)-Salbutamol	616 (261–1452)	0·35
(±)-Salbutamol	581 (348–969)	0·68	(—)-α-Ethyl-noradrenaline	1100 (760–1591)	1
(±)-α-Ethylisoprenaline	1009 (783–1301)	0·77	(±)-α-Ethyl-isoprenaline	2320 (1558–3456)†	1
(—)-α-Ethylnoradrenaline	1360 (1159–1597)	1	(±)-Soterenol	2876 (1069–7735)	0·26
N-Methyldopamine	6302 (4061–9779)	0·8	(—)-Phenylephrine	5588 (848–36 834)	0·05
(±)-α-Methyldopamine	8200 (3418–19 700)	0·53	*N*-Methyldopamine	11 157 (7848–15 863)†	0·63
Dopamine	11 665 (7354–18 503)	0·6	(±)-Synephrine	21 165 (6331–70 760)	0·14
(—)-Phenylephrine	14 283 (2967–68 760)	0·43	Dopamine	22 126 (4032–121 430)	0·26
(±)-Synephrine	23 230 (9356–57 675)	0·53	(±)-α-Methyldopamine	44 605 (10 233–194 440)	0·56

Significant differences between equiactive concentration ratios for tension and rate within each agonist, as determined by Student's *t*-test, are depicted as $*P < 0.02$ and $\dagger P < 0.05$. Absence of a symbol indicates no significant difference. RIA = Relative Intrinsic Activity.

Table 2. *Regression and correlation coefficients calculated from the regression analysis of equiactive molar concentration ratios of sympathomimetic amines relative to (−)-isoprenaline on tension ($log_e x$) and rate ($log_e y$).* Analyses were performed on the entire series or the full and partial agonists alone.

	Regression coeffcient	Correlation coefficient
All agonists	1·08 (0·91–1·26)	0·96 (0·89–0·99)
Full agonists*	1·20 (1·09–1·32)	0·99 (0·97–0.99)
Partial agonists	0·57 (—0·69–1·83)	0·81 (—0·68–1·00)

95% confidence limits in brackets.
* Those with RIA values greater than 0·5 but including dopamine.

regression coefficients for both plots are unity. There was an improved correlation coefficient of 0·99 which suggests that the weaker partial agonists may influence the analysis. This was borne out when the remaining agonists were examined alone, which yielded poor correlation and regression coefficients as shown in Table 2.

Antagonism of the positive chronotropic and inotropic responses to (−)-isoprenaline by propranolol, practolol, atenolol and acebutolol was assessed by calculating pA_2 values (Table 3). The order of potency was propranolol > acebutolol > atenolol > practolol on both parameters and there was little difference between pA_2 values for rate and tension for each antagonist. The slopes of the regression plots of close to unity confirmed that the

antagonism was competitive and that the experimental design was adequate.

DISCUSSION

Are the positive chronotropic and inotropic responses of the heart mediated via different β_1-adrenoceptors? From both agonist and antagonist studies Dreyer & Offermeier (1975) hold the view that they are. Their experiments using a range of full and partial agonists showed examples of chronotropic selectivity particularly with increased *N*-substitution in a series of phenylephrine derivatives. This trend was not found in the present study. Dreyer & Offermeier (1975) claimed that any indirect activity of the sympathomimetic amines did not alter their conclusions since the result was unaffected by treatment of the atria with cocaine. However it is known that the storage (Angelakos, Fuxe & Torchiana, 1963) and release (Blinks, 1966; Goldberg, 1972) of noradrenaline varies between left and right atria. Giudicelli (1975) has also found differences between rate and tension in a limited selection of agonists, but admits that since no control of the pharmacological environment was made no valid conclusion can be drawn.

Table 3. *pA_2 values for the antagonism of the positive inotropic and chronotropic responses to (−)-isoprenaline by various β-adrenoceptor antagonists.*

	Increased tension		Increased rate	
	pA_2	slope	pA_2	slope
Acebutolol	7·33	0·82	7·03	1·02
Atenolol	6·98	1·11	7·16	0·87
Practolol	6·73	0·94	6·70	0·94
Propranolol	8·43	0·99	8·79	0·86

Slope values are for the regression line of log (DR-1) against log molar antagonist concentration.

In the present study the possibilities of indirect activity, neuronal and extraneuronal uptake and stimulation of α-adrenoceptors have been excluded by using reserpine-pretreated animals and phenoxybenzamine incubation. Furthermore, it can confidently be assumed that the measured responses were mediated via β-adrenoceptors since the shifts of dose-response curves by practolol for a number of the sympathomimetic amines have been shown to be of the same order (unpublished observations). Under these controlled conditions only four selective agonists were evident from comparisons of equiactive molar concentration ratios relative to the (−)-isoprenaline reference. (−)-Noradrenaline and its (±)-α-methyl derivative were selective for tension, whereas (±)-α-ethylisoprenaline and *N*-methyldopamine were rate selective. Several observations suggest, however, that although significant, these were merely isolated examples of selectivity and that the β-adrenoceptors subserving the rate and tension responses could not be separated.

1. No apparent structural trends for the four examples of selectivity emerged. This contrasts with the consistent structural pattern that led to the subdivision of β_1- and β_2-adrenoceptors, which arose from the observation that α-substitution of adrenaline (Palm, Langeneckert & Holtz, 1967) or isoprenaline (Lands & others, 1967) produced an increased selectivity for the bronchiolar and vascular β-adrenoceptors.

2. If a compound exhibited partial agonist activity it did so generally on both rate and tension, although the degree of such activity differed between these parameters. The only exceptions were (±)-α-ethylisoprenaline and (−)-orciprenaline which were full agonists on tension and nearly so on rate. If two distinctly different receptors were involved, one might expect partial agonism on one parameter but not on the other. Salbutamol is a partial agonist here but is a full agonist on bronchial β_2-adrenoceptors (Farmer & others, 1970; Buckner & Abel, 1974).

3. Any gross difference between receptor subtypes should be revealed by the use of a simple range of full agonists having the catecholamine nucleus (Furchgott, 1972). The relative order of activity isoprenaline > noradrenaline > adrenaline found here for the rate and tension responses characterizes the receptors for both these responses of the guinea-pig atria as the β_1-type (Furchgott, 1967; Arnold & McAuliff, 1971; Grana, Lucchelli & Zonta, 1974). To determine whether the rank orders of potency were identical on both parameters, regression analyses were performed on the entire series of compounds and just the full agonists. That they were identical was indicated by the approximately unity values for the correlation coefficients. However, identical rank orders alone are not sufficient to deduce that the same receptor types are involved, since correlation coefficients of unity have been obtained when the β_1-adrenoceptors of the heart were compared with β_2-adrenoceptors of bronchiolar smooth muscle (Apperley, Daly & Levy, 1976). More important for differentiation were the considerably differing regression coefficients. In the present analysis these were approximately unity for the entire series and the full agonists alone, indicating that the equiactive molar concentration ratios for rate and

tension—and therefore the receptors— are identical.

In a parallel study performed in these laboratories a similar range of 15 sympathomimetic amines have been examined in tissues controlled pharmacologically in the same way, but without the internal isoprenaline reference. This study was considered less satisfactory because of its dependence upon between-tissue variation, but it too yielded correlation and regression coefficients of approximately unity (unpublished observations).

Antagonists have been used in the present study and elsewhere to determine whether the β_1-adrenoceptors responsible for the positive chronotropic and inotropic responses differ. Using pA_2 values, Dreyer & Offermeier (1975) claimed that several β-adrenoceptor antagonists preferentially blocked the positive chronotropic responses. This situation however can arise due to differential neuron uptake when noradrenaline is the agonist (Lumley & Broadley, 1975) and may explain their results, although they failed to state the agonist used. In contrast, Fenyvesi (1972) found preferential blockade of the inotropic responses in cat atria. Blinks (1967) also observed that the positive inotropic responses to isoprenaline were antagonized by propranolol to a greater extent than the positive chronotropic responses, but concluded that the difference was too small to warrant the proposal that the responses were mediated through different receptor types. This conclusion was supported by Bristow & Green (1970) who obtained equipotent blockade of isoprenaline-induced force and rate responses in rabbit atria.

In the present study we have measured the antagonism of rate and tension responses by propranolol, acebutolol, atenolol and practolol as the pA_2 values. Using (−)-isoprenaline as the agonist to avoid interference from differential neuronal uptake, the pA_2 values for rate and tension did not differ with any of the antagonists.

In conclusion, the evidence from both the agonist and antagonist studies suggests that the β_1-adrenoceptors mediating the positive chronotropic and inotropic resonses do not warrant further sub-classification into two separate groups.

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REFERENCES

AHLQUIST, R. P. (1948). *Am. J. Physiol.*, **153**, 586–600.
ANGELAKOS, E. T., FUXE, K. & TORCHIANA, M. L. (1963). *Acta physiol. scand.*, **59**, 184–192.
APPERLEY, G. H., DALY, M. J. & LEVY, G. P. (1976). *Br. J. Pharmac.*, **57**, 235–246.
ARNOLD, A. & MCAULIFF, J. P. (1971). *Archs int. Pharmacodyn. Thér.*, **193**, 287–293.
ARUNLAKSHANA, O. & SCHILD, H. O. (1959). *Br. J. Pharmac. Chemother.*, **14**, 48–58.
BLINKS, J. R. (1966). *J. Pharmac. exp. Ther.*, **151**, 221–235.
BLINKS, J. R. (1967). *Ann. N.Y. Acad. Sci.*, **139**, 673–685.
BOISSIER, J. R., GIUDICELLI, J. F., LARNO, S. & ADVENIER, C. (1973). *Eur. J. Pharmac.*, **22**, 141–149.
BROADLEY, K. J. & LUMLEY, P. (1977). *Br. J. Pharmac.*, **59**, 51–60.
BRISTOW, M. & GREEN, R. D. (1970). *Eur. J. Pharmac.*, **12**, 120–123.
BUCKNER, C. K. & ABEL, P. (1974). *J. Pharmac. exp. Ther.*, **189**, 616–625.
DREYER, A. C. & OFFERMEIER, J. (1975). *Pharmac. Res. Comm.*, **7**, 151–161.
FARMER, J. B., KENNEDY, I., LEVY, G. P. & MARSHALL, R. J. (1970). *Ibid.*, **22**, 61–63.
FENYVESI, T. (1972). *Kisérl. Orvostud.*, **23**, 53–86.
FURCHGOTT, R. F. (1967). *Ann. N.Y. Acad. Sci.*, **139**, 553–570.
FURCHGOTT, R. F. (1972). In: *Handbook of Experimental Pharmacology*, vol. 33, pp. 283–335. Editors: Blaschko, H. and Muscholl, E., Berlin: Springer-Verlag.
GIUDICELLI, J. F. (1975). *J. Pharmac. (Paris)*, **6**, 221–236.
GOLDBERG, L. I. (1972). *Pharmac. Rev.*, **24**, 1–29.
GOVIER, W. C. (1968). *J. Pharmac. exp. Ther.*, **159**, 82–90.
GRANA, E., LUCCHELLI, A. & ZONTA, F. (1974). *Il Farmaco Ed. sci.*, **29**, 786–792.
JEWITT, D., BIRKHEAD, J., MITCHELL, A. & DOLLERY, C. (1974). *Lancet*, **2**, 363–367.
LANDS, A. M., ARNOLD, A., MCAULIFF, J. P., LUDUENA, F. P. & BROWN, T. G. (1967). *Nature*, **214**, 597–598.
LUMLEY, P. & BROADLEY, K. J. (1975). *Eur. J. Pharmac.*, **34**, 207–217.
LUMLEY, P., BROADLEY, K. J. & LEVY, G. P. (1977). *Cardiovasc. Res.*, **11**, 17–25.
PALM, D., LANGENECKERT, W. & HOLTZ, P. (1967). *Naunyn-Schmiedebergs Arch. Pharmac.*, **258**, 128–149.
TUTTLE, R. R. & MILLS, J. (1975). *Circulation Res.*, **36**, 185–196.