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Assessment of the effectiveness of β-adrenoceptor blocking agents towards cardiac and bronchiolar responses of the pithed guinea-pig to electrical stimulation of the spinal outflow

D. T. BURDEN AND M. W. PARKES

Pharmacology Department, Roche Products Ltd., Welwyn Garden City, Herts., England

Summary

1. The responses of heart rate and resistance to lung inflation of the pithed guinea-pig on electrical stimulation of the thoracic spinal roots could be related to similar responses to injected catecholamines, such that dose-stimulus frequency relations could be plotted.

2. The range of frequency of stimulation that was equi-effective with a dose range of injected catecholamines was higher for effects on air overflow than for heart rate. The slope of the relations for heart rate also differed from that for air overflow. These features may reflect a difference in effectiveness of the sympathetic innervation of heart and bronchial tree.

3. Propranolol was equally effective in reducing the responses of heart rate and air overflow to injected noradrenaline. Practolol was somewhat more active against the effects of noradrenaline on air overflow than on heart rate, though equally active against the effects of isoprenaline.

4. For the assessment of equivalent blockade of the effects of cord stimulation on heart rate and air overflow, frequency-ratios corresponding to a noradrenaline dose-ratio of 2 were derived from the slopes of the dose-frequency relations; for air overflow this value was approximately 2 and for heart rate approximately 1·4.

5. When the doses required to produce these degrees of blockade were computed from the dose-response relations for blockade of the effects of cord stimulation by propranolol, they were found to be similar for effects on heart rate and air overflow. For practolol, the effective dose for block of heart rate increase was found to be lower than that for air overflow.

Introduction

In a previous paper (Burden, Parkes & Gardiner, 1971) we reported that in the pithed guinea-pig, in which the thoracic spinal outflow was stimulated electrically via the pithing rod, propranolol was more effective in reducing the effect of this upon the bronchoconstriction due to methacholine than in reducing the accompanying tachycardia, whereas practolol reduced both effects at the same dose. As we remarked, these findings do not reflect clinical reports of the relative selectivity of these two drugs.

The effects of blocking agents upon the responses of different organs to stimuli applied in this way to the sympathetic nervous system must depend, not only upon the relative effectiveness of the agents in blocking the receptors involved at each site, but also upon factors such as the degree of innervation of each organ, the effectiveness of the applied stimulation for each and the amounts of transmitter released as the result.

An attempt was therefore made to re-determine the relative effectiveness of β-adrenoceptor blocking agents for the cardiac and bronchiolar consequences of cord stimulation by calibrating the effects of this stimulation against the effects of injected amines and comparing the activity of the blocking agents towards both.

Part of this work was included in a preliminary communication to the British Pharmacological Society at their Oxford meeting in September 1971 (Bainbridge, Burden & Parkes, 1971).

Methods

Guinea-pigs were pithed under urethane anaesthesia (2·5–3 g/kg intraperitoneally) and the spinal nerve roots stimulated electrically in the thoracic region, as described previously (Burden *et al.*, 1971). Square pulses of 80 V and 5 ms duration were used at various frequencies for periods of 20 seconds. Responses other than β-adrenoceptive were blocked by giving D-tubocurarine, 1 mg/kg, and phenoxybenzamine, 2 mg/kg; doses of methacholine were used to induce an increase in air overflow. All drugs were injected intravenously.

Drugs used were: (±)-propranolol hydrochloride and (±)-practolol (I.C.I.), methacholine (Koch-Light), D-tubocurarine chloride (Burroughs Wellcome), phenoxybenzamine hydrochloride (Smith, Kline & French), urethane (Koch-Light), (−)-adrenaline (B.D.H.), (−)-noradrenaline bitartrate (Koch-Light), (−)-isoprenaline sulphate (Burroughs Wellcome).

Results

Electrical stimulation and injected amines

Both the increase in heart rate and reduction of methacholine-induced bronchoconstriction showed regression with frequency of electrical stimulation. Since the duration of stimulation was always 20 s, this amounted to a regression on number of pulses. A comparison of the effects of the delivery of 20 pulses at different frequencies (Table 1) showed no such dependence of effect on frequency.

Figure 1 shows the relations found for the increase in heart rate with stimulus frequency (a) and with dose of injected noradrenaline (b); the envelopes indicate the computed 95% confidence limits. Figure 2 similarly shows the relations for effects upon methacholine-induced bronchoconstriction. These were expressed as

TABLE 1. *The effects of cord stimulation with* 20 *pulses of* 80 *V and* 50 *ms width at various frequencies upon heart rate and increase in air overflow induced by methacholine,* 12 *μg/kg, intravenously, in three pithed guinea-pigs*

Frequency (Hz)	Increase in heart rate (beats/min)			% Reduction of increase in air overflow		
	1	2	3	1	2	3
0·5			70			24
1	80	55	80	43	15	27·5
2	80	55		54	37	
5	70	45		60	21	

the ratio of the doses of methacholine required for an equal increase of air overflow before and after electrical stimulation (a) or a dose of injected noradrenaline (b) and were plotted as (dose-ratio−1) on a logarithmic scale.

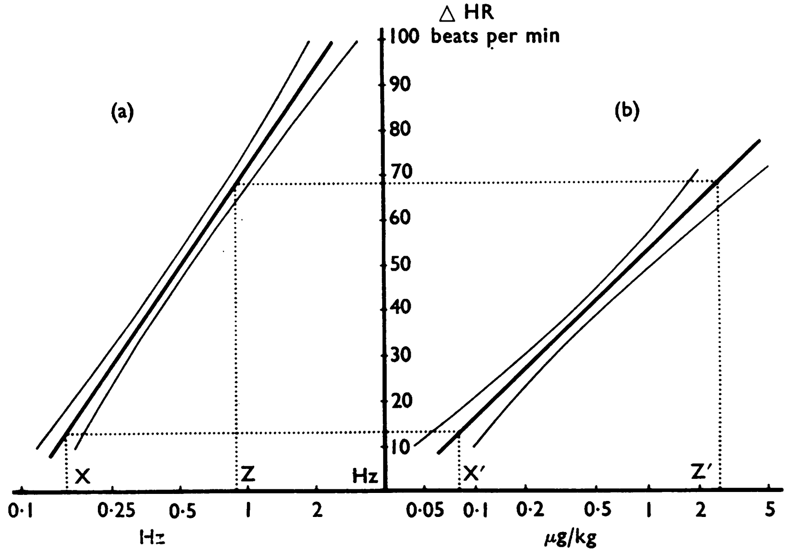

FIG. 1. Regression lines computed from the increase in heart rate (ΔHR) caused by (a) thoracic cord stimulation with 5 ms pulses of 80 V at various frequencies for 20 s and (b) intravenous doses of noradrenaline, in the pithed guinea-pig. The envelopes enclose the 95% confidence limits. For (a) b=73·7±S.E. 4·9, n=55; (b) b=36·8±S.E. 3·45, n=55.

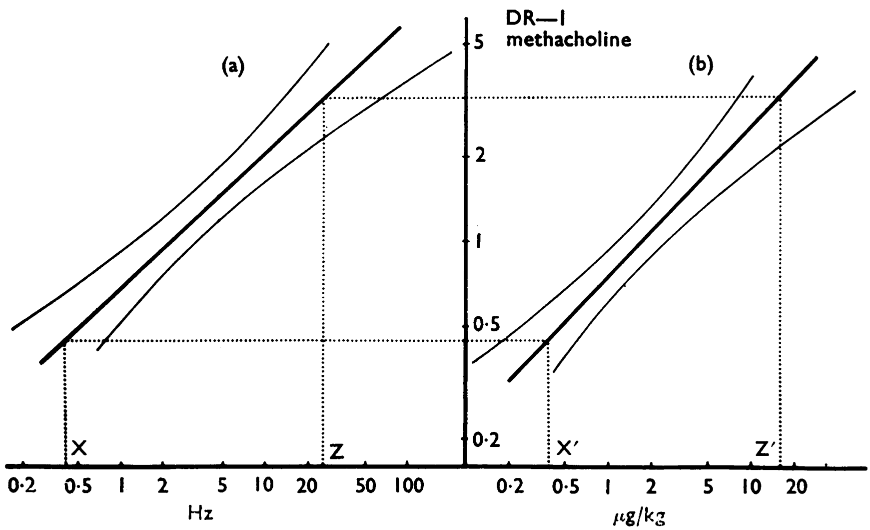

FIG. 2. Regression lines computed from the increase in dose of intravenously injected methacholine required to produce a given increase in air overflow (expressed as dose ratio−1) in the pithed guinea-pig when (a) the thoracic cord is stimulated with 5 ms pulses of 80 V at various frequencies for 20 s or (b) various doses of noradrenaline are injected intravenously. The envelopes enclose the 95% confidence limits. For (a) b=0·48±S.E. 0·073, n=37; (b) b=0·53±S.E. 0·08, n=30.

From these and similar relations determined for adrenaline and isoprenaline, further relations could be derived (Fig. 3) between stimulus frequency and dose of each of the catecholamines for equal effect upon heart rate or methacholine-induced increase in air overflow. It will be seen that the catecholamines were effective for heart rate and air overflow over similar dose ranges, though the relative activities of the three amines were different in the two situations. For heart rate, isoprenaline>noradrenaline=adrenaline, in the approximate ratio 10:1:1, whereas for bronchodilatation, isoprenaline>adrenaline>noradrenaline, in the approximate ratio 40:10:1. These relative activities are in general agreement with those reported by Lands & Brown (1964) from studies with isolated organs.

Further, it will be seen that the range of stimulus frequency that matched doses of the catecholamines for equal effect was greater with air overflow than with heart rate; indeed, the two ranges barely overlap. Finally, it should be noted that the slopes of the two sets of relations differ, being steeper for effects on air overflow than for tachycardia; the relations for all the catecholamines for either effect are, however, similar.

Although the relations shown in Fig. 3 have been derived by plotting doses and stimulus frequencies for equal effect, from the primary relations such as those shown in Figs. 1 and 2, their slopes can, of course, be obtained as ratios of the slopes of the latter. These are shown in Table 2, which also gives their 95% confidence limits, computed by means of the Behrens-Fisher analogue of Fieller's theorem (Finney, 1964).

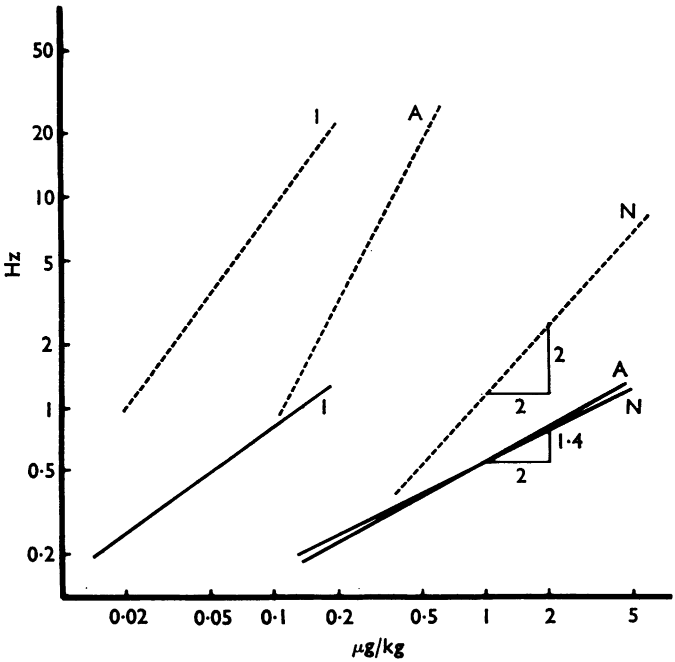

FIG. 3. Relationships between the intravenous doses of catecholamines and the frequency of stimulation of the thoracic cord of the pithed guinea-pig with 5 ms pulses of 80 V for 20 s for equal effect upon —— heart rate and - - - - the dose-ratio of methacholine to produce a given increase in air overflow, plotted from the computed regression lines such as those shown in Figs. 1 and 2. For example, X plotted against X′, Z against Z′ (Figs. 2 & 3). I=Isoprenaline, A=adrenaline, N=noradrenaline.

Antagonism by β-adrenoceptor blocking agents

Noradrenaline

The antagonism of β-adrenoceptor blocking agents towards the effects of injected amines was determined from the ratio of the doses of the amine required for equal effect in increasing heart rate or in reducing methacholine-induced bronchoconstriction, before and after a dose of the blocking agent. This ratio was expressed as (dose-ratio—1) and plotted against the dose of blocking agent, both on logarithmic scales.

Figure 4 shows the relations computed from results for practolol against the effects of noradrenaline on heart rate and air overflow; the computed doses required for 50% antagonism (DR−1=1) by propranolol and practolol are given in Table 3. Here it will be seen that whereas propranolol antagonized both effects of noradrenaline in similar doses, practolol was more active against effects on heart rate than on air overflow by a factor of 2·5. When computed by the method set

TABLE 2. *Characteristics of the primary relations between dose of injected catecholamine or frequency of electrical stimulation of the cord and increase in heart rate or reduction of methacholine-induced air overflow in pithed guinea-pigs; and of the slopes of the secondary relations between equi-effective dose and stimulus frequency derived therefrom*

	Heart rate			Air overflow		
	n	b	95% Confidence limits	*n*	b	95% Confidence limits
Stimulus frequency	55	73·7	63·9–83·4	37	0·48	0·33–0·63
Noradrenaline	55	36·8	29·9–43·7	30	0·53	0·37–0·69
Dose v. frequency		0·5	0·39–0·63		1·1	0·71–1·72
Adrenaline	34	40·5	32·0–49·0	18	0·92	0·71–1·13
Dose v. frequency		0·55	0·43–0·69		1·92	1·31–2·94
Isoprenaline	14	52·4	33·8–70·9	12	0·65	0·37–0·94
Dose v. frequency		0·71	0·46–0·98		1·35	0·75–2·23

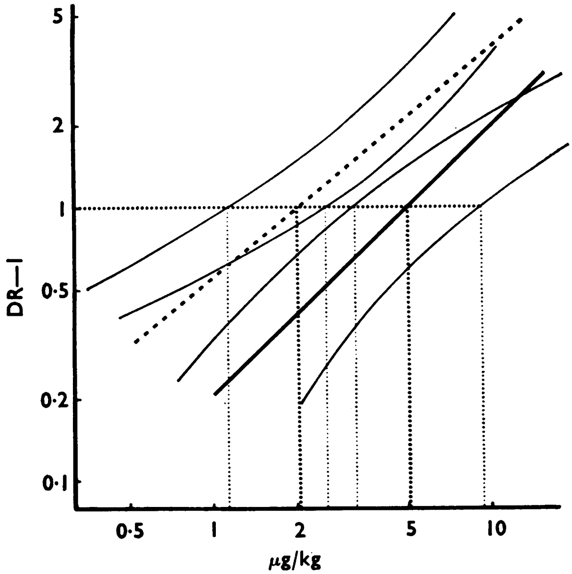

FIG. 4. Regression lines computed from the increase, produced by intravenous doses of practolol in the intravenous dose of noradrenaline required for a given increase in heart rate (——) or reduction in methacholine-induced bronchoconstriction (- - - -), plotted as dose-ratio−1. The envelopes enclose the computed 95% confidence limits. For effects on heart rate, b=0·97±S.E. 0·22, n=9. For effects on air overflow, b=0·83±S.E. 0·13, n=8. Computed doses producing blockade corresponding to DR=2 are given in Table 3.

out in the Appendix to a recent paper by Conolly, Davies, Dollery & George (1971) this difference was significant at the 1% level.

Isoprenaline

Figure 5 shows that against the effects of injected isoprenaline on heart rate and air overflow, practolol was antagonistic in similar doses, as seen also from the computed doses for 50% block given in Table 3.

Electrical stimulation

Antagonism of the blocking agents towards the effects of cord stimulation on heart rate and air overflow was expressed in a corresponding fashion to that used for injected amines, namely, the ratio of frequencies for equal effect before and after antagonist, expressed as (frequency-ratio—1) on a logarithmic scale. Figures 6 and 7 show these relations for propranolol and practolol, respectively, and it

TABLE 3. *Doses for 50% blockade by propranolol and practolol of the effects of injected noradrenaline or isoprenaline on heart rate and increase of air overflow induced by methacholine in pithed guinea-pigs*

	Dose for 50% blockade, μg/kg intravenously, with 95% confidence limits	
	Propranolol	Practolol
Noradrenaline		
Heart rate	3·65 (2·4–5·3)	5·0 (3·1–7·9)
Air overflow	2·3 (1·4–3·8)	2·0 (1·3–3·1)
Isoprenaline		
Heart rate	—	11·4 (5·2–19·5)
Air overflow	—	20·9 (8·1–37·4)

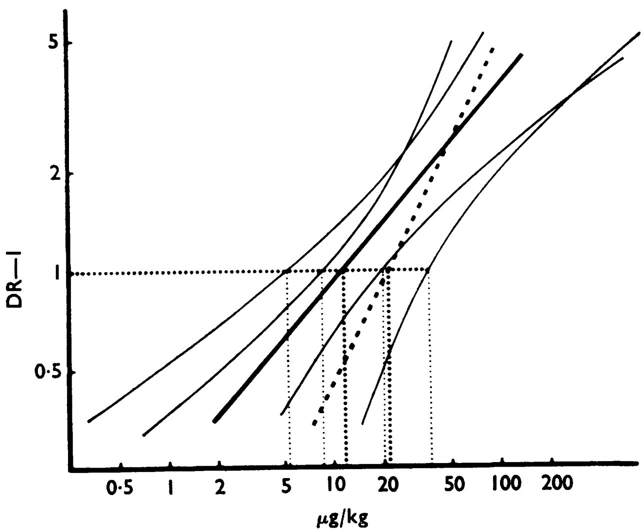

FIG. 5. Regression lines computed from the increase, produced by intravenous doses of practolol, in the intravenous dose of isoprenaline required for a given increase in heart rate (——) or reduction in methacholine-induced bronchoconstriction (- - - -), plotted as dose-ratio−1. The envelopes enclose the computed 95% confidence limits. For effects on heart rate, b=0·59±S.E. 0·11, n=17. For effects on air overflow, b=1·01±S.E. 0·27, n=12. Computed doses producing blockade corresponding to DR=2 are given in Table 3.

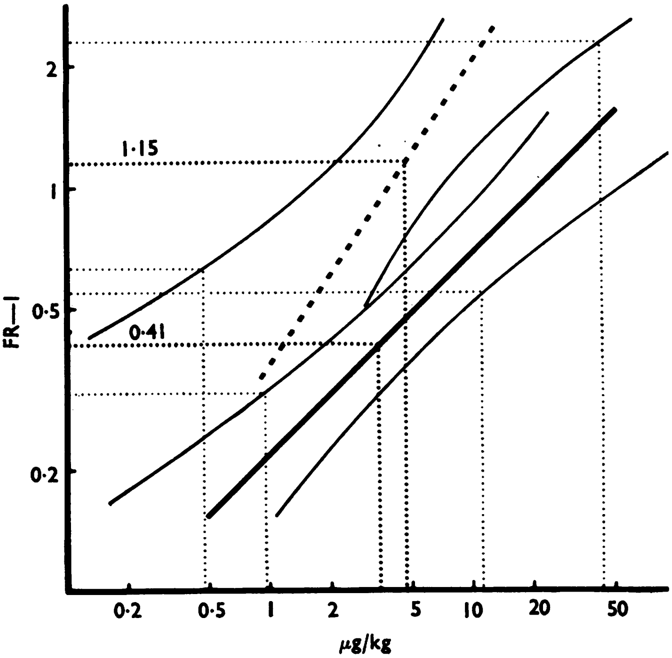

FIG. 6. Regression lines computed from the increase, produced by intravenous doses of propranolol, in the frequency of thoracic cord stimulation of the pithed guinea-pig (with 5 ms pulses of 80 V for 20 s) required for a given increase in heart rate (——) or reduction in methacholine-induced bronchoconstriction (- - - -), plotted as frequency-ratio−1. The envelopes enclose the computed 95% confidence limits. For effects on heart rate, b=0·49± S.E. 0·076, n=18. For effects on air overflow, b=0·74±S.E. 0·18, n=9. Intercepts are shown corresponding to frequency-ratios equivalent to DR=2 for injected noradrenaline, and their computed 95% confidence limits (see **Discussion**) and the computed doses producing blockade corresponding to these values are given in Table 4.

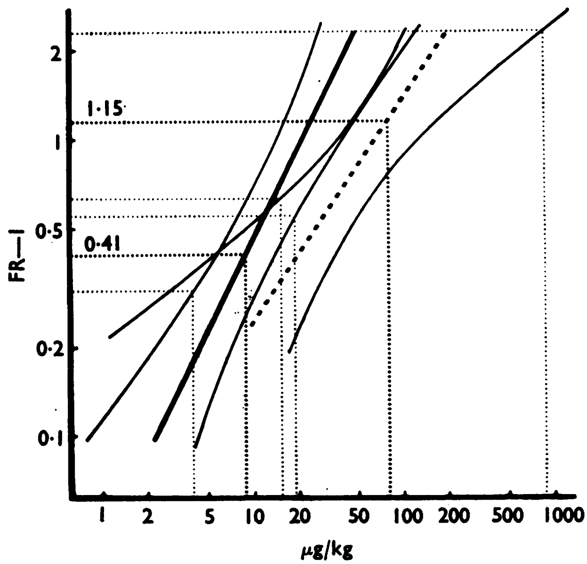

FIG. 7. Regression lines computed from the increase, produced by intravenous doses of practolol, in the frequency of thoracic cord stimulation of the pithed guinea-pig (with 5 ms pulses of 80 V for 20 s) required for a given increase in heart rate (——) or reduction in methacholine-induced bronchoconstriction (- - - -), plotted as frequency-ratio−1. The envelopes enclose the computed 95% confidence limits. For effects on heart rate, b=1·04± S.E. 0·16, n=8. For effects on air overflow, b=0·75±S.E. 0·175, n=12. Intercepts are shown corresponding to frequency-ratios equivalent to DR=2 for injected noradrenaline, and their computed 95% confidence limits (see **Discussion**) and the computed doses producing blockade corresponding to these values are given in Table 4.

appears that propranolol was more active against the effect of stimulation on air overflow than on heart rate, whereas practolol shows a tendency to greater effectiveness against tachycardia than against bronchodilatation.

Discussion

The greater effectiveness of electrical stimulation of the thoracic spinal roots for heart rate than air overflow, relative to effective doses of catecholamines, suggests a difference in effective sympathetic innervation between the heart and the bronchiolar tree of the kind postulated as the basis for this study. Moreover, the frequency of 10 Hz for a duration of 30 s that was used in the previous experiments (Burden *et al.*, 1971) was almost certainly supramaximal for heart rate and thus is likely to have given a misleading estimate of the effectiveness of the β-adrenoceptor blocking agents in this respect, relative to their action on air overflow, for which the stimulus frequency used was appropriate.

The selection of noradrenaline with which to compare the effectiveness of cord stimulation for heart rate and air overflow rests on two assumptions: first, that injected noradrenaline is similarly effective and similarly susceptible to block by antagonists, at the two sites concerned; and second, that cord stimulation is essentially a means of delivering noradrenaline to those sites with a relative effectiveness reflected in the responses. The first of these assumptions is unverifiable since the parameters of the two responses studied are not comparable, but it is supported by the similar dose ranges in which noradrenaline was found to evoke both kinds of response; and further by the similar effectiveness of propranolol in blocking each. The second is in accordance with current physiological concepts, although the dependence of response level upon the number of stimuli delivered is at variance with the results of electrical stimulation of sympathetic nerve fibres in the cat, in which the amount of noradrenaline released varied with the frequency of application of a fixed number of shocks (Brown & Gillespie, 1957; Brown, Davies & Gillespie, 1958).

The shallower relation between stimulus frequency and equi-active dose of catcholamine found for effects on heart rate than for bronchodilator effects suggests a real difference in stimulus-response relations at the sites concerned. This makes it necessary to consider the appropriate levels of effect at which to compare the effectiveness of blocking agents at these sites.

Since the pairs of relations shown in Figs. 4 and 5 are acceptably parallel, it is valid to compare the activities shown by an antagonist against the actions of injected amines at the two sites, by the doses required for a given degree of antagonism, expressed by the dose-ratio of amine. In Figs. 4 and 5, this is illustrated by the dose for 50% blockade, i.e., that for a dose-ratio of 2 (DR-1=1), and the computed values, with 95% confidence limits, are shown in Table 3.

For cord stimulation, however, the relations shown in Fig. 3 indicate that a dose-ratio of 2 for injected amine corresponds to a different frequency-ratio for each kind of response; about 2 for air overflow but approximately 1·4 for heart rate. More precisely, the figure may be calculated from the expression:

$$\text{antilog } (m \log 2)$$

where m is the ratio of the slopes of the primary relations (Table 2). The figures thus obtained are:

For effects on heart rate 1·41 (1·31–1·55).

For effects on air overflow 2·15 (1·63–3·29).

When these frequency-ratios (in the form FR-1) are used to compare the effectiveness of propranolol and practolol against responses to cord stimulation in the relations shown in Figs. 6 and 7, it may be seen that the doses corresponding to equal degree of blockade for heart rate and air overflow by propranolol approach each other closely. In the case of practolol, however, the corresponding dose for blockade of the effect on air overflow is 9 times that for heart rate (Table 4).

TABLE 4. *Doses for blockade by propranolol and practolol of the effects of cord stimulation that are equivalent to those blocking by 50% the effects of noradrenaline on heart rate and increase of air overflow induced by methacholine in pithed guinea-pigs*

	Propranolol		Practolol	
	Dose μg/kg	Range*	Dose μg/kg	Range*
Heart rate	3·6	1·0–11·3	9·3	4·0–19·1
Air overflow	4·7	0·5–44·2	80·2	15·2–895

*Range derived by computation of the intercepts between the computed 95% confidence limits of the appropriate frequency-ratio (see **Discussion**) and of the regression lines for blockade (see Figs. 6, 7).

The doses corresponding to these FR intercepts are, however, subject to two sorts of uncertainty; that represented by the confidence limits of the estimates of blocking activity, shown as the envelopes in Figs. 6 and 7, and that represented by the confidence limits of FR given above. These sources of error impose fairly wide limits on the estimated effective doses for equivalent blockade of the two responses. Nevertheless, the values shown in Table 4 confirm that practolol affects the tachycardia due to cord stimulation in smaller doses than are required for an equivalent effect on air overflow, despite its marginally greater activity against injected noradrenaline in its effect upon air overflow.

In this respect, therefore, the results of this study may indicate the degree of selectivity exercised by the drugs towards the part played by the sympathetic nervous sysem in maintaining heart rate and bronchiolar diameter.

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