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The assessment of β-adrenoceptor blocking potency and cardioselectivity *in vitro* and *in vivo*

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The β-adrenoceptor blocking potencies and cardioselectivities (versus tracheal smooth muscle) of propranolol, practolol and M & B 17803A have been assessed on *in vitro* and *in vivo* guinea-pig preparations. Propranolol was found to be potent but non-selective, practolol to be less potent but cardioselective. M & B 17803A resembled practolol in potency but had a more modest degree of cardioselectivity *in vivo*. These observations suggest that the procedures used form a simple scheme which permits a probably relevant assessment of β-adrenoceptor blocking potency and cardioselectivity.

A desirable feature of β-adrenoceptor blocking agents intended for use in the treatment of angina pectoris is a pronounced action on β_1-adrenoceptors in the heart accompanied by little or no effect on β_2-adrenoceptors in bronchial smooth muscle (Dollery, Paterson & Conolly, 1969; Nickerson, 1970). Practolol possesses such a preferential or cardioselective action (Barrett, Crowther & others, 1968; Dunlop & Shanks, 1968) and since its introduction, the need to assess the potency and cardioselectivity of β-adrenoceptor blocking agents has increased. Assessments have been made from effects on *in vitro* guinea-pig preparations of cardiac muscle and tracheal smooth muscle (Åblad, Brogård & others, 1970; Farmer & Levy, 1970); on dog or cat heart rate *in vivo* and guinea-pig tracheal smooth muscle *in vitro* (Dunlop & Shanks, 1968; Baum, Rowles & others, 1971); on dog heart rate and perfused hind limb *in vivo* (Robson & Kaplan, 1970; Basil, Jordan & others, 1971) and from effects on guinea-pig heart rate and anaphylactic bronchospasm *in vivo* or on cat heart rate and diastolic pressure *in vivo* (Basil & others, 1971). In view of the variety of procedures and species used, it would appear that there is no generally accepted scheme for determining the potencies and cardioselectivities of β-adrenoceptor blocking agents. Those that have been used do not permit assessment to be made on *in vitro* and *in vivo* preparations from the same species nor do they all employ an indicator of lung function.

We have developed a simple procedure based on guinea-pig *in vitro* and *in vivo* preparations. The *in vivo* preparation offers the advantage of permitting the effects of β-adrenoceptor blocking agents on cardiac muscle and tracheal smooth muscle to be measured simultaneously in an intact animal. The validity of the procedure has been examined with propranolol, practolol and M & B 17803A [(±)-1-(2-acetyl-4-n-butyramidophenoxy)-2-hydroxy-3-isopropylamino propane HCl].

METHODS

Guinea-pig in vitro *preparations*

Male Tuck guinea-pigs, 500–800 g, were killed by a blow on the head and the hearts and tracheae removed.

Spontaneously-beating right atria. Spontaneously-beating right atria were dissected from the hearts and suspended under 1 g tension in a water-jacketed 15 ml tissue bath at 37–38° containing physiological salt solution, of the following composition (g/litre): NaCl, 6·9; $NaHCO_3$, 2·1; glucose, 2·0; $CaCl_2$, 0·27; KCl, 0·35; KH_2PO_4, 0·16; $MgSO_4$, 0·14, bubbled with 5% carbon dioxide in oxygen. Responses were recorded by an Ether 2oz strain gauge. The method of determining β-adrenoceptor blocking potency was basically that of Bristow, Sherrod & Green (1970). After allowing 30 min for the equilibration of the system, a cumulative dose-response curve for the chronotropic action of isoprenaline was determined. When the maximum rate of beating was reached, the tissue was washed repeatedly for 20 min. The lowest concentration of antagonist was added to the bath 20 min before commencing the second dose-response curve to isoprenaline, a longer equilibration period having been found unnecessary. This was repeated with two further doses of antagonist, doubling the concentration each time.

pA_2 values were determined from graphs of log (x–1) versus negative log molar concentration of antagonist (Bristow & others, 1970).

Isolated intact trachea preparation. Tracheae were mounted in water-jacketed 80 ml organ baths as described by Farmer & Coleman (1970). After 30 min, a cumulative dose-response curve for the inhibition by isoprenaline of the increases in intraluminal pressure produced by electrical stimulation (7 s at 20 Hz, 7–10 V and 1 ms every 60 s) was determined. When the inhibition had reached a maximum, the tissue was washed repeatedly for 20 min and a dose of antagonist added 20 min before the second dose-response curve to isoprenaline. Only one dose of antagonist could be evaluated on each preparation. Consequently, the pA_2 values were determined from graphs constructed from results from three preparations.

In vitro *guinea-pig cardioselectivity ratio.* The selective action of the β-adrenoceptor blocking agent on cardiac tissue as against tracheal smooth muscle was assessed by taking the antilogarithm of the difference between the mean pA_2 values obtained from the atria and tracheae.

Guinea-pig in vivo *preparation*

Male Tuck guinea-pigs, 500–800 g, were anaesthetized with pentobarbitone sodium (60 mg/kg intraperitoneally), and ventilated artificially using a Starling miniature respiration pump. Blood pressure was recorded from one carotid artery by a Bell and Howell blood pressure transducer, heart rate by a Devices Ratemeter and intraluminal pressure from an isolated segment of trachea (Lynn James, 1969) by a Statham low pressure transducer. All drugs were administered into the jugular vein.

Isoprenaline (0·5 μg) produced a simultaneous increase in heart rate and decrease in tracheal intraluminal pressure and was given every 15 min, each dose being followed after 5 min by the administration of 3 to 6 mg pentobarbitone sodium to maintain a constant level of anaesthesia (Fig. 1). When control responses to isoprenaline were established, a dose of β-adrenoceptor blocking agent was given 5 min before the next isoprenaline challenge. This cycle of events—β-adrenoceptor blocking agent, isoprenaline challenge, and pentobarbitone sodium—was repeated every 15 min, doubling the dose of antagonist each time, until the heart rate and tracheal intraluminal pressure responses to the isoprenaline challenge were reduced to less than 50% of the control values (Fig. 1).

β-Adrenoceptor blocking agents caused varying degrees of bradycardia in this preparation. This was taken into account in the calculation of the inhibition of the

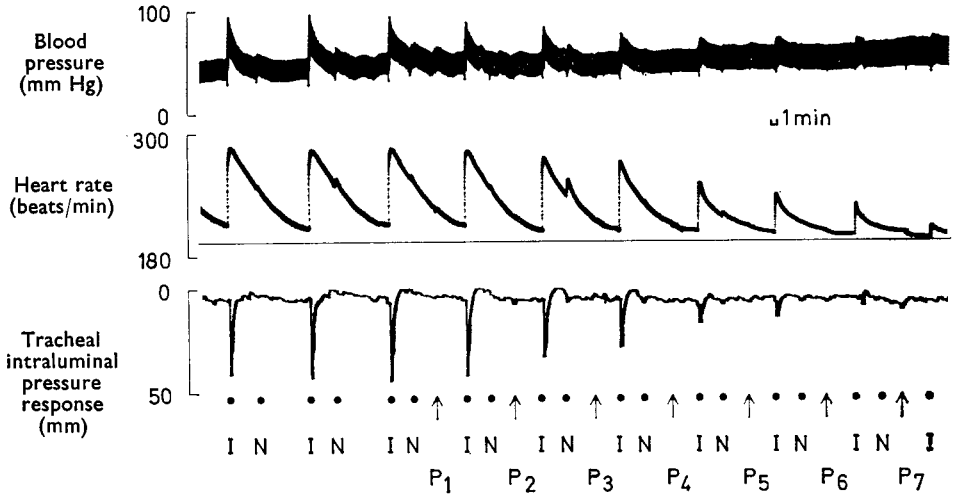

FIG. 1. Recording of blood pressure, heart rate and tracheal intraluminal pressure of an anaesthetized guinea-pig showing the effects of increasing doses (mg/kg) of propranolol (P—see Fig. 2 for doses) on the responses to 0·5 μg isoprenaline (I). 3 mg pentobarbitone sodium (N) administered every 15 min to maintain anaesthesia.

heart rate response to isoprenaline by taking the lowest heart rate observed and measuring each heart rate response in beats/min from this level (Fig. 1). No correction was found to be necessary for the tracheal intraluminal pressure responses which were simply measured in mm trace length. In both cases, the degree of inhibition produced by each dose of β-adrenoceptor blocking agent was calculated as a % of the mean control response. The mean % inhibitions from five to seven experiments were plotted against the doses of β-adrenoceptor blocking agent (Fig. 2). From these

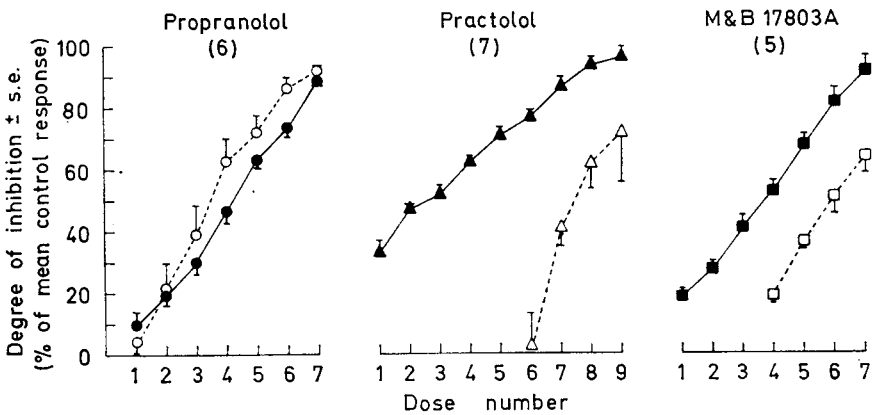

FIG. 2. Dose-response relations for the effects of propranolol, practolol and M & B 17803A on the isoprenaline-induced heart rate (closed symbols, solid lines) and tracheal intraluminal pressure (open symbols, broken lines) responses of anaesthetized guinea-pigs. Each point is the mean of the number of experiments shown in brackets and the vertical lines represent the standard errors of the mean.

	Dose number								
	1	2	3	4	5	6	7	8	9
Propranolol (mg/kg)	0·0075	0·015	0·03	0·0625	0·125	0·25	0·5		
Practolol (mg/kg)	0·25	0·5	1	2	4	8	16	32	64
M & B 17803A (mg/kg)	0·125	0·25	0·5	1	2	4	8		

graphs, ED50 values were obtained for the heart rate and tracheal intraluminal pressure responses.

In vivo *guinea-pig cardioselectivity ratio.* The selective action of the β-adrenoceptor blocking agent on cardiac tissue as against tracheal smooth muscle was assessed by dividing the ED50 for inhibition of the tracheal intraluminal pressure response by the ED50 for inhibition of the heart rate response.

Drugs

Isoprenaline hydrochloride; M & B 17803A (a gift from May and Baker); pentobarbitone sodium (Nembutal, Abbott); practolol and propranolol (gifts from ICI). All drugs were dissolved in 0·9% w/v sodium chloride solution and the doses reported refer to the salts.

RESULTS

Guinea-pig in vitro *spontaneously-beating right atria and isolated intact trachea preparations*

The β-adrenoceptor blocking potencies and cardioselectivity ratios of propranolol, practolol and M & B 17803A are shown in Table 1. Each pA_2 is the mean ± s.e. of

Table 1. *β-Adrenoceptor blocking potency and cardioselectivity of propranolol, practolol and M & B 17803A on guinea-pig* in vitro *preparations.* Each pA_2 value is the mean ± s.e. of the results of the number of log (x–1) versus negative log molar concentration of antagonist graphs shown in brackets. Cardioselectivity ratio is obtained from the antilogarithm of the difference between the mean pA_2 values obtained from atria and tracheae.

β-Adrenoceptor blocking agent	pA_2 value right atrium	pA_2 value trachea	Cardioselectivity ratio
Propranolol	8·35 ± 0·04 (4)	8·17 ± 0·11 (4)	1·5
Practolol	7·09 ± 0·21 (4)	6·18 ± 0·07 (4)	8·1
M & B 17803A	7·21 ± 0·11 (5)	6·40 ± 0·05 (4)	6·5

four or five values. Propranolol, with pA_2 values of 8·35 and 8·17 for spontaneously-beating right atria and isolated intact tracheae respectively, was the most potent compound studied. It was approximately equiactive on both tissues as the cardioselectivity ratio of 1·5 shows. M & B 17803A giving atrial and tracheal pA_2 values of 7·21 and 6·40 respectively, was less active than propranolol on both preparations. However, it was 6·5 times more active on cardiac muscle than on tracheal smooth muscle. Practolol, with atrial and tracheal pA_2 values of 7·09 and 6·18 respectively, was the least potent compound but displayed the greatest cardioselectivity, being 8·1 times more active on cardiac muscle than on tracheal smooth muscle.

Table 2. *β-Adrenoceptor blocking potency and cardioselectivity of propranolol, practolol and M & B 17803A on the* in vivo *guinea-pig preparation.* Each value is obtained from the mean of the number of experiments shown in brackets (see Fig. 2). Cardioselectivity ratio is obtained by dividing the ED50 for inhibition of tracheal intraluminal pressure response by the ED50 for inhibition of heart rate response.

β-Adrenoceptor blocking agent	ED50 heart rate response (mg/kg)	ED50 tracheal intraluminal pressure response (mg/kg)	Cardioselectivity ratio
Propranolol (6)	0·073	0·044	0·6
Practolol (7)	0·8	21·8	27·3
M & B 17803A (5)	0·86	4·0	4·7

Guinea-pig in vivo *preparation*

The β-adrenoceptor blocking potencies and cardioselectivity ratios of propranolol, practolol and M & B 17803A derived from Fig. 2 are shown in Table 2. Each ED50 was obtained from the means of from five to seven experiments. Propranolol, with ED50 values of 0·073 and 0·044 mg/kg for inhibition of the heart rate and tracheal intraluminal pressure responses respectively, was the most potent compound. In contrast to the guinea-pig *in vitro* preparations, it was more active on tracheal smooth muscle than on cardiac muscle as the cardioselectivity ratio of 0·6 shows. Practolol, with heart and tracheal ED 50 values of 0·8 and 21·8 mg/kg respectively, was less potent than propranolol on both preparations. However, it displayed considerable cardioselectivity, being 27·3 times more active on cardiac muscle than on tracheal smooth muscle. M & B 17803A, with heart and tracheal ED50 values of 0·86 and 4·0 mg/kg respectively, was slightly less effective than practolol in inhibiting the heart rate response but more active than practolol in inhibiting the tracheal intraluminal pressure response. Consequently, it was less cardioselective than practolol being only 4·7 times more active on cardiac muscle than on tracheal smooth muscle.

DISCUSSION

The results obtained from the guinea-pig *in vitro* preparations indicate that in terms of β-adrenoceptor blocking potency and cardioselectivity, propranolol is potent but non-selective and practolol is less potent but cardioselective. Thus, although the absolute pA_2 values observed are lower than those reported by others (Bristow & others, 1970; Farmer & Levy, 1970), the conclusions that can be drawn from them confirm the generally accepted views about the β-adrenoceptor blocking potencies and cardioselectivities of these drugs (Barrett & others, 1968; Brick, Hutchison & others, 1968; Dunlop & Shanks, 1968; Macdonald & McNeill, 1968). The data obtained from the *in vivo* guinea-pig preparation also indicate that propranolol is potent but non-selective and practolol is less potent but cardioselective and so substantiate the observations on the *in vitro* preparations.

That practolol appears more cardioselective *in vivo* than *in vitro* suggests that this known effect of the drug is more easily detected in the former situation than in the

latter. Moreover, although propranolol is usually considered to be non-selective, *in vivo* it has a slightly greater effect on tracheal smooth muscle than on cardiac muscle which agrees with its effects on lung and heart adenyl cyclase, the enzyme considered to be an integral part of the β-adrenoceptor (Burges & Blackburn, 1972). Consequently, within the proposed screen, the guinea-pig *in vivo* preparation is considered to provide the more reliable assessment of the potencies and cardioselectivities of β-adrenoceptor blocking agents.

Although M & B 17803A was slightly more active *in vitro* and slightly less active *in vivo* than practolol on cardiac tissue, the overall impression is of a compound with β-adrenoceptor blocking activity similar to that of practolol but with a more modest degree of cardioselectivity, particularly *in vivo*.

The observed potency of M & B 17803A is consistent with that reported from experimental animals and man (Basil & others, 1971; Cuthbert & Owusu-Ankomah, 1971). Moreover, that it possesses cardioselectivity in the guinea-pig *in vitro* and *in vivo* preparations agrees with observations in other animals (Basil & others, 1971).

Thus, the results with propranolol, practolol and M & B 17803A suggest that the proposed scheme provides an adequate assessment of β-adrenoceptor blocking potency and cardioselectivity.

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