J. Pharm. Pharmac., 1973, **25**, 621–628 *Received February 5* ,1973

The evaluation of the α-adrenoceptor blocking action of indoramin, phentolamine and thymoxamine on the rat and guinea-pig isolated mesenteric vasculature and aortic spiral preparations

M. G. COLLIS AND B. J. ALPS

Wyeth Institute of Medical Research, Huntercombe Lane South, Taplow, Maidenhead, Berkshire, U.K.

The perfused mesenteric vasculature of the rat is a specific, sensitive and stable preparation for the rapid determination of α-adrenoceptor blocking pA_2 values. Both types of pA_2 determination—the direct determination (Schild, 1947) and the Arunlakshana & Schild (1959) analysis—can be made on this tissue. Indoramin, phentolamine and thymoxamine were evaluated and gave respectively α-block pA_2 values of 8·05, 7·84 and 6·47. The pA_2 values made on the rat perfused mesentery were compared with those obtained on the rat aorta (indoramin 7·68, phentolamine 8·29, thymoxamine 6·50), the guinea-pig aorta (indoramin 7·38, phentolamine 7·64, thymoxamine 6·93) and the perfused mesenteric bed of the guinea-pig (indoramin 8·48, phentolamine 7·51, thymoxamine 6·97). Statistical analyses of Kb (dissociation constant) values suggest that indoramin has an additional blocking action on resistance vessels, which is not possessed by phentolamine or thymoxamine.

The isolated strip preparation (Furchgott & Bhadrakom, 1953) of the guinea-pig or rabbit aorta which is commonly used for the evaluation of α-adrenoceptor blocking drugs has two major disadvantages when employed in screening procedures. First, the aorta contributes little, functionally, to the *in vivo* peripheral resistance of the systemic vasculature, and second, its responses to exogenous noradrenaline are slow and require a 10–15 min wash-out time.

Peripheral resistance is known to be elevated in hypertension (Freis, 1960) and therefore it is preferable that novel antihypertensive agents be evaluated on a vascular bed containing resistance vessels (arterioles) such as the rat mesenteric vasculature. The perfused mesenteric bed of the rat has been used to study vascular reactivity in experimental hypertension (McGregor & Smirk, 1968; Haeusler & Haefely, 1970; Finch, 1971; Haeusler & Finch, 1972).

This paper describes the use of the perfused rat mesentery for the rapid evaluation of α-adrenoceptor blocking drugs, including the hypotensive agent indoramin (Archibald, 1968). The results are compared with values for α-adrenoceptor blockade made on the rat and guinea-pig isolated aortae and the guinea-pig isolated perfused mesenteric vasculature.

METHODS

Rat perfused mesenteric vasculature preparation

The technique used for the perfusion of the mesenteric vasculature is a modification of that described by McGregor (1965).

Female rats (220–250 g) were anaesthetized by intraperitoneal injection of 60 mg kg^{-1} pentobarbitone sodium. The abdomen was opened by mid-line incision and the superior mesenteric artery cannulated using a Portex cannula (o.d. 0·75 mm). The caecal, ileo-colic, colic, and pancreatico-duodenal branches of the artery were ligated. The perfused mesentery was severed close to the ileum, and the arterial system placed in a water-jacketed glass cup at 37°. Krebs solution, bubbled with 5% carbon dioxide in oxygen was perfused at 2 ml min^{-1} by a peristaltic pump. Agonist drugs (0·02–0·2 ml) were injected at 2 min intervals through a rubber tube proximal to the cannula. Constrictor responses were recorded as changes in perfusion pressure by a Bell and Howell (4–327–L221) pressure transducer and written out on a Devices M.2. recorder.

The guinea-pig isolated perfused mesenteric vasculature preparation

Male guinea-pigs (500–750 g) were used, the general technique was similar to that described for the rat mesenteric preparation. The guinea-pigs were more difficult to anaesthetize with intraperitoneal pentobarbitone sodium than rats. The technique of intra-thoracic injection of the anaesthetic (30 mg kg^{-1}) proved to be efficient. A perfusion fluid flow rate of 2·6 ml min^{-1} and a perfusion cannula with a terminal o.d. of 1·02 mm were used. The perfusion rate and cannula size were necessarily larger than those used for the rat.

Rat or guinea-pig isolated aortic spiral preparation (see Furchgott & Bhadrakom, 1953)

Experiments on aortic spirals were made using Krebs solution at 37° and bubbled with 5% carbon dioxide in oxygen. Organ bath volumes of 38 ml were used and ascorbic acid (10^{-4}M) was added to prevent oxidation of noradrenaline. This concentration of ascorbic acid was without effect on the contractile mechanism of the tissues used.

Contractions of the thoracic aorta were recorded by a variable inductance transducer (0·5 g load) connected to a phase discriminator and pen recorder. Cumulative dose-response curves were obtained for noradrenaline, added to the bath every 5 min.

In all experiments, antagonist equilibrium with the tissue was judged to have occurred when constant agonist responses were elicited.

Analysis of results

Determination of pA_2 values

In each experiment, the mean dose-ratio was calculated from measurements (Gaddum, Hameed & others, 1955) taken at various corresponding points on the log dose-response lines obtained in the presence and absence of the antagonists. This ratio was then subjected to analysis as described by Arunlakshana & Schild (1959) and pA_2 values were determined graphically. The pA_2 values of indoramin on the rat mesentery were also obtained using the direct method of Schild (1947).

The dissociation constants of the antagonists from the α-adrenoceptors (Kb) were calculated from the formula:—

$$Kb = \frac{\text{Molar concentration of antagonist}}{\text{Agonist dose-ratio} - 1} \quad \text{or}$$

$$Kb = \frac{B}{(x - 1)} \text{mol litre}^{-1}$$

Drugs used

Ascorbic acid (BDH), indoramin hydrochloride (Wyeth), (±)-isoprenaline sulphate (Burroughs Wellcome), (—)noradrenaline bitartrate (Koch-Light), papaverine sulphate (BDH), phentolamine mesylate (Ciba), thymoxamine hydrochloride (Warner). All drug concentrations are expressed in terms of base.

RESULTS

The rat perfused mesenteric vasculature preparation

The baseline perfusion pressure

The mean pressure recorded before cannulation of the tissue was 47·1 ± 3·8 mm Hg (n = 24) which was mainly due to the narrow bore of the cannula (the compliance of the perfusion system).

The mean baseline pressure of the cannulated tissue was 74·2 ± 3·0 mm Hg (n = 24). The 27·0 mm Hg rise in perfusion pressure on cannulation of the tissue corresponds with the 25 mm Hg increment found by Malik & Ling (1969) even though these workers used a 25 ml min^{-1} flow rate.

Isoprenaline (0·5 – 1·0 μg) and papaverine (1 μg) reduced the baseline perfusion pressure by 2–4 mm Hg, indicating that the tissue is virtually atonic, the resistance to flow being due to the calibre and elasticity of the mesenteric arterioles.

The stability of the preparation

The baseline perfusion pressure remained constant for at least 6 h. The tissue showed stable sensitivity to noradrenaline after about 1 h of dosing with this agonist and remained stable for 4–5 h.

The sensitivity of the preparation

The tissue usually gave recordable constrictor responses at 0·01 – 0·02 μg of noradrenaline, the maximum response being reached at 8·0 – 16·0 μg.

The pressor responses of the rat mesenteric vasculature to doses of noradrenaline are shown in Fig. 1. The responses were extremely rapid, enabling a 2 min dose-cycle to be employed.

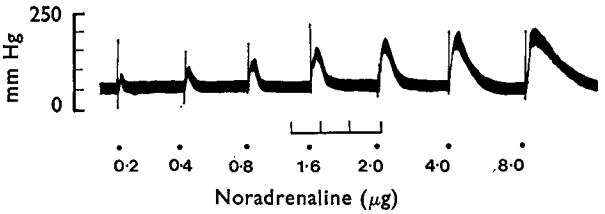

FIG. 1. Noradrenaline-induced constrictor responses (mm Hg) of the rat isolated perfused mesenteric vasculature. The horizontal axis represents minute intervals. Note the initial injection artifact followed by a rapid constrictor response which returns to a stable baseline.

The specificity of the preparation

The linearity and unit slopes of the "Arunlakshana and Schild" plots for phentolamine and thymoxamine (Table 1), demonstrate that noradrenaline is specifically activating α-adrenoceptors in the rat mesenteric vascular preparation. Tachyphylaxis to 5-hydroxytryptamine (continuous infusion of $0{\cdot}57 \times 10^{-5}$M) had no effect on responses to noradrenaline. This lack of cross-tachyphylaxis was taken as evidence for noradrenaline activating α-adrenoceptors, and not a combination of α- and 5-hydroxytryptamine receptors.

The direct α-block pA_2 determination of indoramin

The direct determination of the α-adrenoceptor blocking activity of indoramin gave the following results: mean α-block $pA_2 = 8{\cdot}05 \pm 0{\cdot}15$ (s.e.; $n = 10$).

The Arunlakshana and Schild analysis of indoramin α-block pA_2

Using the calculated mean dose-ratio (x) of noradrenaline from each experiment, the log $(x-1)$ was plotted against the negative log of the molar concentration of indoramin. The calculated regression line (slope – 1·06) intersected the abscissa at the pA_2 value of 8·05. The experimental $pA_2 - pA_{10}$ value was 0·85.

The pA_2 value was higher than that previously reported (Alps, Hill & others, 1972) for experiments made on the guinea-pig isolated aorta. To determine whether a species or a tissue variation caused this apparent difference in potency, α-block pA_2 values were also determined on the rat isolated aorta and the guinea-pig isolated perfused mesentery. Phentolamine and thymoxamine were also evaluated for comparison with indoramin. The results of complete Arunlakshana & Schild (1959) analyses for the three drugs are given in Table 1.

Table 1. *The results of Arunlakshana and Schild analyses of the α-block pA_2 values for indoramin, phentolamine and thymoxamine on the perfused mesenteric vessels of the rat and guinea-pig and the rat and guinea-pig isolated aortic spiral preparations.*

Tissue	Antagonist	Concentration (M) min	Concentration (M) max	n	Slope	pA_2
Perfused mesenteric vessels of the rat	Indoramin	5×10^{-8}	5×10^{-6}	21	−1·06	8·05
	Phentolamine	5×10^{-8}	10^{-5}	14	−0·91	7·84
	Thymoxamine	5×10^{-7}	3×10^{-5}	16	−1·00	6·47
Perfused mesenteric vessels of the guinea-pig	Indoramin	10^{-8}	5×10^{-6}	17	−1·03	8·48
	Phentolamine	10^{-7}	10^{-5}	13	−0·93	7·51
	Thymoxamine	5×10^{-7}	3×10^{-5}	12	−0·93	6·97
Rat isolated aortic spiral	Indoramin	10^{-7}	10^{-5}	13	−0·85	7·68
	Phentolamine	10^{-7}	10^{-5}	15	−0·91	8·29
	Thymoxamine	5×10^{-7}	3×10^{-5}	13	−1·11	6·50
Guinea-pig isolated aortic spiral	Indoramin (Alps & others 1972)	10^{-7}	10^{-4}	16	−1·16	7·38
	Phentolamine	10^{-7}	10^{-5}	14	−0·90	7·64
	Thymoxamine	10^{-7}	10^{-5}	12	−1·02	6·93

The mean log (dose ratio – 1) values for each antagonist on each tissue lie on their respective regression lines.

The guinea-pig isolated perfused mesenteric bed

The sensitivity to noradrenaline of the guinea-pig isolated perfused mesenteric bed was similar to that of the rat mesenteric vasculature. Tissues were very sensitive to noradrenaline giving initial pressor responses to 0·02 μg of noradrenaline, and reaching a maximum of 100–140 mm Hg at doses of 0·8–8·0 μg.

The mean baseline pressure of the perfusion system used was 13 mm Hg, a lower value than that used for the rat mesentery because of the wider terminal cannula employed.

The mean perfusion pressure of the tissue was 37 mm Hg. An increase in pressure of 24 mm Hg occurred on cannulation of the guinea-pig superior mesenteric artery. The increase in pressure on cannulation of the same artery in the rat caused a 27 mm Hg rise in pressure. The analogous increase in pressure on cannulation would suggest that the diameter and/or compliance of the mesenteric vessels from the two species was similar.

The results of complete "Arunlakshana and Schild" analyses for indoramin, phentolamine and thymoxamine are given in Table 1.

The rat isolated aortic spiral preparation

The rat isolated aortic spiral is highly sensitive to noradrenaline, as previously reported by Valette & Ngun-Ba-Muoi (1962). In the present study it was found to be approximately 100 times more sensitive to noradrenaline than the guinea-pig aorta.

The rat aorta has spontaneous tone (Redleaf & Tobian, 1958) a property which the guinea-pig aorta does not possess. Concentrations of 10^{-5}M indoramin lowered the inherent tone of the rat aorta by an amount equivalent to 60% of the maximum noradrenaline response (n = 2). However, the maximum response of the relaxed tissue to noradrenaline was identical to the control response. Neither phentolamine nor thymoxamine decreased the tone of the rat aorta at the concentration used.

The results of complete "Arunlakshana and Schild" analyses for indoramin, phentolamine and thymoxamine are given in Table 1.

The guinea-pig aortic spiral preparation

The results of complete "Arunlakshana and Schild" analyses for indoramin (Alps & others, 1972), phentolamine and thymoxamine are given in Table 1.

Statistical analysis of results

The Kb (dissociation constant) values were calculated for each dose-ratio used in the determination of the pA_2 value for each drug on each tissue. The mean Kb values (± s.e.) are given in Table 2, with *t*-test significance levels.

DISCUSSION

A perfused rat mesenteric vasculature preparation has been described which reacts rapidly with and shows high sensitivity and stability to noradrenaline.

The speed of response of this perfused preparation has proved useful for the determination of the direct α-block pA_2 value for indoramin, and the results from the direct method are identical to those calculated from the complete Arunlakshana & Schild (1959) form of analysis. The constrictor responses of the tissue to noradrenaline

Table 2. *Kb values* $\times$ 10^9 *mol litre*$^{-1}$ *for indoramin, phentolamine and thymoxamine on the aorta and mesenteric vessels of the rat and guinea-pig. Mean Kb values* $\times$ 10^9 *mol litre*$^{-1}$ *are given* $\pm$ *s.e.: number of experiments (n) in parentheses. Students' t-test significance level values are given for each drug between tissues of the same species.*

	Guinea-pig		Rat	
Drug	Aorta	Mesentery	Aorta	Mesentery
Indoramin Kb	40·7 ± 11·5 (n = 12)	3·95 ± 0·92 (n = 17)	44·2 ± 7·27 (n = 12)	9·12 ± 1·37 (n = 22)
Significance level	0·01		0·001	
Phentolamine Kb	36·4 ± 4·1 (n = 13)	45·3 ± 7·3 (n = 13)	9·84 ± 1·62 (n = 15)	41·02 ± 14·88 (n = 14)
Significance level	Not significant		0·05	
Thymoxamine Kb	89·6 ± 14·0 (n = 12)	152·8 ± 20·0 (n = 12)	290·0 ± 120·0 (n = 13)	408·0 ± 55·0 (n = 16)
Significance level	0·05		Not significant	

were caused by α-adrenoceptor activation and not by a combination of α- and 5-hydroxytryptamine receptors.

Indoramin, phentolamine and thymoxamine were competitive α-adrenoceptor blocking agents on the isolated aorta and mesenteric vasculature of both the rat and the guinea-pig.

Thymoxamine was consistently the least potent antagonist; phentolamine was the most potent on the aorta, while indoramin was most potent on the mesentery. Since small arteries and arterioles and not larger vessels (e.g. aorta) are responsible for peripheral resistance, it is likely that indoramin would be a more effective *in vivo* hypotensive agent than phentolamine, due to its superior vasodilator potency on resistance vessels. Previous studies (Alps & Rashid, unpublished experiments) have demonstrated that indoramin is a more potent hypotensive agent in the anaesthetized cat than phentolamine. Phentolamine has β-adrenoceptor stimulatory actions on the heart (Zahir & Gould, 1971) while indoramin is cardioinhibitory (Alps, Johnson & Wilson, 1970) and these different actions on the heart would also influence the relative hypotensive potencies of the two agents.

Indoramin at 10^{-5}M was found to relax the rat aorta, but the height of the maximum noradrenaline-induced contraction was unchanged. This result could suggest a direct vasodilator action of indoramin at high concentration, perhaps due to its local anaesthetic activity (Alps, Hill & others, 1970). Phentolamine has been reported to have a direct action on blood vessels (Taylor, Sutherland & others, 1965) but in the experiments described in the present study, concentrations up to 10^{-5}M did not cause any relaxation of the rat aorta. The factors involved in the maintenance of tone in the rat aorta are not fully known, but spontaneous spike potentials have been recorded in mammalian large arteries which could be associated with basal tone (Keatinge, 1968). If action potentials in the rat aorta are associated with its inherent tone then it is likely that a drug with local anaesthetic properties would stabilize the muscle cell membranes and reduce the tone.

The pA_2 results indicate that indoramin is a more potent α-adrenoceptor antagonist on the mesentery than the aorta, and phentolamine more potent on the aorta than the mesentery. For accurate investigation of antagonist-receptor interactions, pA_2 values alone are insufficient. Kb values were therefore calculated since the dissociation constant for one antagonist reacting with receptors of the same type should be constant (Furchgott, 1967). This constancy should hold irrespective of the tissue in which the receptors are found, providing that diffusion barriers or agonist degradation and removal rates do not vary between the tissues.

Considering indoramin, the difference between Kb values on the aorta and mesentery was highly significant for both species. The Kb values made on (a) the aorta of the two species, and (b) on the mesentery of the two species were in the same order of magnitude. It would appear from the results that indoramin has a significantly more potent action on vessels in the mesentery than on the aorta. Since this potency difference occurred in the tissue of both the guinea-pig and the rat, it cannot be due to a species difference in sensitivity to indoramin. Sensitivity changes in the aorta cannot account for indoramin's more potent action on the mesentery since neither phentolamine nor thymoxamine showed this consistent potency difference. The significant difference between Kb values for indoramin on the aorta and mesentery must reflect, either a different α-adrenoceptor type which is blocked by indoramin and not by phentolamine or thymoxamine, or some property of indoramin which renders it more potent on resistance vessels than the other two drugs.

With phentolamine, there was no significant difference between Kb values on the guinea-pig aorta and mesentery. The Kb value found on the rat mesentery was also close to those determined for guinea-pig tissues. However, the Kb value (and pA_2) on the rat aorta was significantly different from that found on the rat mesentery. Comparison with results previously reported by Clineschmidt, Geller & others (1970), would suggest that the pA_2 values for phentolamine described in this paper on guinea-pig tissues are unusually low, rather than the pA_2 on the rat aorta being unusually high.

With thymoxamine, there was no significant differences between Kb values on the aorta and mesenteric vasculature of the rat but dissociation constants made on the guinea-pig aorta and mesenteric vasculature were significantly different at the 5% level. This difference in Kb values was due to variations in the dose-ratios used to compute the dissociation constant value, the pA_2 values were extremely close and no real difference in potency was apparent. Comparing rat and guinea-pig tissues, thymoxamine appeared to be more potent on rat tissues than on those of the guinea-pig. Birmingham & Szolcsanyi (1965) did not use any rat tissues in their evaluation of the α-blocking potency of thymoxamine but found pA_2 values of 7·20 (guinea-pig aortic strip), 6.90 (rabbit aortic strip) and 7·01 (dog carotid artery strip). The species difference in response to thymoxamine cannot be explained at present, but since there is no real difference between Kb values on tissues from the same species, the receptors involved in the aorta and mesentery would appear to be identical.

It is interesting to speculate that indoramin may have additional affinity for arteriolar smooth muscle, unrelated to α-blockade. It has already been shown that indoramin does not affect vascular smooth muscle in a conventional non-specific manner, as judged by its lack of effect in antagonising constrictor responses induced by angiotensin (Alps & others, 1972). However, coupled with α-adrenoceptor blockade, an

additional activity of indoramin on arteriolar vascular smooth muscle might well indicate that this drug could be usefully employed in the treatment of peripheral vascular disorders.

Acknowledgements

The authors wish to acknowledge the helpful suggestions made by Dr. E. S. Johnson in reading the draft of the paper, and to thank Mr. R. Bridle for technical assistance.

REFERENCES

ALPS, B. J., HILL, M., JOHNSON, E. S. & WILSON, A. B. (1970). *Br. J. Pharmac.*, **40**, 153P

ALPS, B. J., HILL, M., JOHNSON, E. S. & WILSON, A. B. (1972). *Ibid.*, **44**, 52–62.

ALPS, B. J., JOHNSON, E. S. & WILSON, A. B. (1970). *Ibid.*, **40**, 151–152 P.

ARCHIBALD, J. L. (1968). *Chim. Therapeutique*, **3**, 397.

ARUNLAKSHANA, O. & SCHILD, H. O. (1959). *Br. J. Pharmac. Chemother.*, **14**, 48–58.

BIRMINGHAM, A. T. & SZOLCSANYI, J. (1965). *J. Pharm. Pharmac.*, **17**, 449–458.

CLINESCHMIDT, B. V., GELLER, R. G., GOVIER, W. C. & SJOERDSMA, A. (1970). *Eur. J. Pharmac.*, **10**, 45–50.

FINCH, L. (1971). *Br. J. Pharmac.*, **42**, 56–65.

FREIS, E. D. (1960). *Physiol. Rev.*, **40**, 27–50.

FURCHGOTT, R. F. (1967). *Ann. N. Y. Acad. Sci.*, **139**, 553–570.

FURCHGOTT, R. F. & BHADRAKOM, S. (1953). *J. Pharmac. exp. Ther.*, **108**, 129–143.

GADDUM, J. H., HAMEED, K. A., HATHWAY, D. E. & STEPHENS, F. F. (1955). *Q. Jl. exp. Physiol.*, **40**, 49–74.

HAEUSLER, G. & FINCH, L. (1972). *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **272**, 101–116.

HAEUSLER, G. & HAEFELY, W. (1970). *Ibid.*, **266**, 18–33.

KEATINGE, W. R. (1968). *J. Physiol.*, **194**, 169–182.

MALIK, M. U. & LING, G. M. (1969). *Circulation Res.*, XXV, 1–9.

MCGREGOR, D. D. (1965). *J. Physiol.*, **177**, 21–30.

MCGREGOR, D. D. & SMIRK, F. H. (1968). *Am. J. Physiol.*, **214**, 1429–1433.

REDLEAF, P. D. & TOBIAN, L. (1958). *Circulation Res.*, VI, 185–193.

SCHILD, H. O. (1947). *Br. J. Pharmac. Chemother.*, **2**, 189–206.

TAYLOR, S. H., SUTHERLAND, G. R., MACKENZIE, G. J., STAUNTON, H. P. & DONALD, K. W. (1965). *Circulation*, XXXI, 741–754.

VALETTE, G. & NGUN-BA-MUOI. (1962). *Ann. Pharm. Franc.*, **20**, 577–582. Cited by BELL, C. (1971). *Br. J. Pharmac.*, **41**, 711–712.

ZAHIR, M. & GOULD, L. (1971). *J. clin. Pharmac.*, **11**, 197–203.