Br. J. Pharmac. (1973), **48**, 686–692.

Further studies regarding the structure activity relationships of β-adrenoceptor antagonists

E. E. BAGWELL* AND E. M. VAUGHAN WILLIAMS

University Department of Pharmacology, Oxford, England

Summary

1. The ortho (M66,527) and para (M66,368) analogues of 1-t-butylamino-3-(methoxyphenoxy)-2-propanol and para substituted tertiary butylphenoxy-1-*N*-isopropylamine-3 propanol-2 oxalate acid (L8429) were tested in dogs for their β-adrenoceptor blocking activity.

2. M66,527, which contains a methoxy group in the ortho position of the benzene ring, was found to be comparable to propranolol in blocking cardiac and peripheral vascular responses to isoprenaline. Like propranolol, M66,527 was more potent on peripheral receptors.

3. Transference of the methoxy group to the para position (M66,368) reduced the overall potency; however, this compound was found to be relatively cardioselective in that it was 2 to 3·6 times more active in blocking cardiac responses to isoprenaline.

4. The cardioselective properties of the short chain para methoxy substituent were less than those reported for compounds with longer para substitutions (i.e. practolol, para oxprenolol and para alprenolol).

5. L8429, with a tertiary butyl group in the para position, was a weak β-adrenoceptor antagonist without cardioselective properties. A longer, less bulky n-butyl group may provide for a more potent and selective antagonist.

6. The results support the view that the size and site of the substituent on the benzene ring may be of importance in determining the cardioselective potency of β-adrenoceptor antagonists.

Introduction

The development of cardioselective β-adrenoceptor blocking drugs poses a problem in structure-activity relationships which, if better understood, might lead to the development of more therapeutically useful drugs.

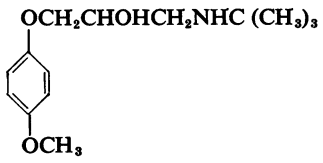
Vaughan Williams & Papp (1970) observed that the cardioselective agent practolol was substituted in the para position of the benzene ring, while oxprenolol and alprenolol, which possess both cardiac and peripheral β-blocking activity, were substituted in the ortho position. These investigators proposed that the point of attachment to the ring might be of importance in determining the cardioselective properties of these compounds and suggested a comparison of the ortho and para analogues of these agents. Subsequent studies (Vaughan Williams, Bagwell & Singh, 1973) revealed that the para analogue of oxprenolol was indeed cardioselective, while the ortho analogue of practolol was not cardioselective. Similar

* On leave from the Department of Pharmacology, Medical University of South Carolina, Charleston, S.C., U.S.A.

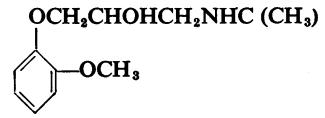
results with ortho and para alprenolol were reported by Åblad, Brogård, Carlsson & Ek (1970).

The present experiments were designed to evaluate further the importance of the ortho and para positions in determining cardioselectivity of β-blocking drugs. It was anticipated that these studies might provide information concerning the nature of a para substituent that might confer even higher cardioselectivity.

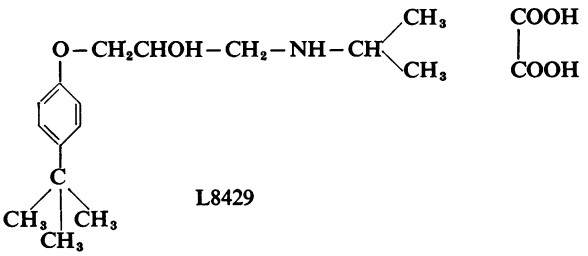
We were fortunate to obtain compounds with a methoxy group in the ortho and para position (M66,527 & M66,368) from Imperial Chemical Industries and one with a tertiary butyl group in the para position (L8429) from Labaz. The structures of these compounds are shown in Figure 1.



M66,368



M66,527



L8429

FIG. 1. Structures of the β-adrenoceptor blocking drugs studied.

Methods

Fifteen dogs anaesthetized with pentobarbitone sodium (30 mg/kg, i.v.) were used in these experiments. An endotracheal tube was inserted and artificial respiration with room air maintained by a Palmer suck-and-thrust pump. Anaesthesia was maintained at a constant level with a continuous infusion of pentobarbitone (5 (mg/kg)/hour). Circulatory reflexes were minimized by severing the vagi and ligating the carotid arteries below the carotid sinus. This procedure has been shown to abolish almost completely reflex responses to hypotension induced by the intravenous administration of 1 mg glyceryl trinitrate (Vaughan Williams *et al.*, 1973). Polyethylene cannulae were inserted into a carotid artery and both jugular veins. The arterial cannula was connected to a pressure transducer (Consolidated Electrodynamics), while the venous cannulae were used for drug administrations.

The aorta was exposed just below the inferior mesenteric artery via a mid-line abdominal incision. The aorta was cleaned and a pre-calibrated flow-transducer (S.E. Laboratories 275) of suitable size placed around it. The inferior mesenteric artery was cannulated in a retrograde direction with a fine polyethylene cannula. The abdominal incision was closed and the cannula and transducer leads brought to the exterior. A left thoracotomy was performed between the fourth and fifth ribs and a pre-calibrated strain gauge arch sutured to the left ventricle for recording

myocardial contractile force. The gauge was placed in a position perpendicular to the interventricular sulcus and the muscle segment beneath stretched approximately 35% (Cotten & Bay, 1956). The chest was closed and heart rate monitored with an electronic tachometer triggered by the strain gauge arch. All measurements were displayed on a Devices M8 recorder. Peripheral blood flow conductance was calculated from simultaneous records of arterial pressure and peripheral aortic blood flow and expressed (ml/min)/mmHg.

The method used for determining cardio-specificity of the blocking agents was as previously described (Vaughan Williams *et al.*, 1973). Intra-arterial injections of small doses (0·01–0·1 μg/kg) of isoprenaline were administered into the inferior mesenteric artery at 4 min intervals with each successive dose being 1·8 times the previous dose. Increasing doses were administered until approximately a three-fold increase in peripheral blood flow was obtained. These small doses of isoprenaline had only minimal effects on heart rate and contractile force. Larger doses of isoprenaline (0·1–1·0 μg/kg) were then administered intravenously and changes in heart rate and contractile force noted. An increase in heart rate of 70 beats per min was taken as the upper desirable limit. The sequence of administration was alternated with each experiment so that in half the animals the intra-arterial injections were made first and in the other half the intravenous injections preceded. When control dose-response curves had been obtained, the β-adrenoceptor blocking agents were administered and the dose-response curves to isoprenaline repeated 15 min later. The doses of isoprenaline were increased until responses equivalent to those of control were obtained. Two or three doses of the blocking drugs were administered in each experiment. The numbers of animals and curves obtained with all drugs studied are summarized in Table 1.

TABLE 1. *Summary of the number of animals and dose-response curves studied with each blocking drug*

Drug	Number of animals	Number of intra-arterial curves	Number of intra-venous curves
M66–527	5	10	10
M66–368	5	16	15
L8429	5	6	5

The data were analysed by plotting log dose-response curves and a least squares regression analysis was used for constructing the curves. The dose of the blocking drug was expressed in log-dose/kg and the responses to isoprenaline were expressed as absolute changes. The dose-response curves in each experiment exhibited an acceptable degree of parallelism, with the implication that the antagonist drugs were acting by competitive blockade. Dose-ratios were determined and pA_2 values for each antagonist calculated.

One of the difficulties which arises when more than one dose of antagonist is used in an *in vivo* experiment is that there is no way to determine accurately the amount of residual activity remaining from the first dose when the second dose is administered. Information relative to this problem was obtained in several experiments by obtaining two consecutive sets of dose-response curves following one dose of the antagonist. It was determined that approximately 50% of the biological activity remained from the first set of curves to the second. Consequently, it appeared reasonable to assume, since successive doses of antagonist drugs were given at hourly intervals, that by adding to each successive dose of antagonist 50% of the previous dose more accurate pA_2 values within animals could be obtained. This hypothesis was subjected to statistical analysis with the New Duncan's Multiple

Range Test (Steel & Touie, 1960). Table 2 summarizes the statistical analyses of the between-dog and within-dog variation for M66,368 and M66,527. It is evident from the high F values that the between-dog variation was much higher than the within-dog variation and would indicate that the adjustment of the antagonist dose to allow for 50% residual activity was a justifiable assumption. Results with L8429 were not included in this analysis. Since this compound was found to be relatively inactive, it was not studied in detail and it was felt that the data points obtained were not sufficient to warrant analysis in this manner.

TABLE 2. *Comparison of between-dog to within-dog variation*

(A)

		Effect		
	Drug	Rate beats/min	Force g	Conductance (ml/min)/mmHg
M66,368	SD between	0·3565	0·2801	1·4930
	SD within	0·2101	0·1520	0·3204
M66, 527	SD between	0·1779	0·2826	0·0817
	SD within	0·0384	0·0422	0·0172

(B)

	Drug	Pooled S.D. of effects	F	$P<$
M66,368	SD between	0·9009	14·975	0·001
	SD within	0·2328		
M66,527	SD between	0·1985	31·45	0·001
	SD within	0·0354		

(A) Summarizes the between-dog and within-dog variation for each parameter studied. (B) Summarizes statistical analysis of the overall between-dog and within-dog variation. Data verify that the between-dog is much greater than the within-dog variance.

Results

Effects of ortho and para methoxy substitution

The ortho substituted methoxy analogue (M66,527) blocked both cardiac and peripheral responses to isoprenaline (Fig. 2A–C ; Table 3). However, the potency of this compound on cardiac β-adrenoceptors was only 1/4 to 1/3 of the observed activity on peripheral receptors (Table 3, column 8).

The para substituted methoxy compound (M66,368) was less active than M66,527 as a β-adrenoceptor blocker (Fig. 2D–F ; Table 3, column 4). In 3 of 5 experiments a dose of 0·2 mg/kg M66,368 failed to block the conductance response to isoprenaline while in one experiment minimal block was obtained with 0·05 mg/kg. However, in contrast to M66,527, the para substituted compound was 2 to 3·6

TABLE 3. *Cardiospecificity of β-adrenoceptor blocking drugs*

		Blockade of chronotropic (C) and inotropic (I) responses to isoprenaline			Blockade of increments in peripheral conductance (P) induced by isoprenaline			
1	2	3	4	5	6	7	8	
Compound	M.W.	pA_2	pA_2 equivalent mol/kg$\times10^{-6}\pm$S.E.	Potency ratio $\frac{C}{I}$	pA_2	PA_2 equivalent mol/kg$\times10^{-6}\pm$S.E.	$\frac{C}{P}$	$\frac{I}{P}$
M66-527	253	C=6·742	C= 0·181±0·034	0·78	7·337	0·046±0·008	0·25	0·32
		I =6·889	I =0·142±0·031					
M66-368	253	C=6·335	C=0·462±0·071**	0·59	6·003	0·994±0·270**	2·15	3·64
		I =6·564	I =0·273±0·062					
L-8429	355·4	C=4·900	C=12·595±4·72*	0·41	5·323	4·720±2·010**	0·37	0·93
		I =5·293	I =5·090±0·454*					

* $P<$ ·001 when compared to M66-527; ** $P<$ ·005 when compared to M66-527.

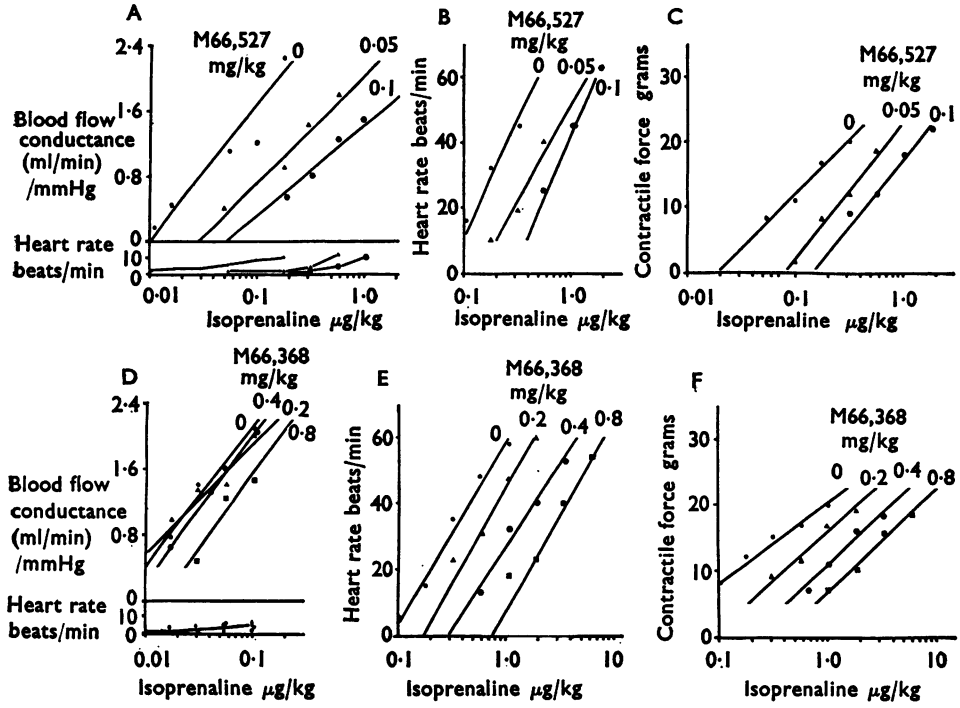


FIG. 2. The effects of M66,527 (panels A–C) and M66,368 (panels D–F) on cardiac and peripheral responses to isoprenaline in anaesthetized dogs. The dose of antagonist is depicted at the top of each dose-response curve. The ordinates show absolute changes in the parameters in response to increasing doses of isoprenaline in μg/kg (abscissae). Isoprenaline was administered intra-arterially (retrograde into the inferior mesenteric artery) in panels A & D and intravenously in panels B, C, E & F. In panels A & D heart rate responses to the intra-arterial injections are also shown.

times more active on cardiac β-adrenoceptors than it was on peripheral receptors (Table 3 column 8).

Effects of para-t-butyl substitution

L8429 exhibited only weak β-adrenoceptor blocking activity with a potency of 1/50 to 1/100 that of the methoxy compounds (Table 3). In addition there was no apparent cardioselectivity associated with its activity (Table 3, column 8).

Intrinsic activity

The intrinsic activity of the β-adrenoceptor blocking compounds studied was minimal. Although there was a trend toward depression of several parameters, these effects were quite variable and in general were not of statistical significance.

Discussion

The present study was initiated to gain further insight into the structural requirements for optimum cardioselectivity in β-adrenoceptor antagonists. Compounds studied included two with short chain (methoxy) substitutions, one in the ortho position (M66,527) and one in the para position (M66,368); along with one com-

pound having a larger tertiary butyl group in the para position (L8429). M66,527 was found to be an antagonist of both cardiac and peripheral β-adrenoceptors with pA_2 values equivalent to those reported for propranolol in an earlier study from this laboratory when the same experimental design was used (Vaughan Williams *et al.*, 1973). This compound, like propranolol, was also found to be more potent on peripheral β-adrenoceptors. Transference of the methoxy group to the para position (M66,368) reduced the overall potency but this compound was found to be relatively cardioselective with a cardiac to peripheral potency ratio of 2 to 3·6 depending on the cardiac parameter used for comparison (Table 3, column 8). However, the cardioselectivity of this para methoxy compound is considerably less than that exhibited by practolol, para oxprenolol (Vaughan Williams *et al.*, 1973) or para alprenolol (Åblad *et al.*, 1970). All of these compounds possess longer chains in the para position than the methoxy substituent of M66,368. These results would suggest that the length of the side chain, as well as the site of attachment to the ring, is important in determining the degree of cardioselectivity inherent in β-adrenoceptor antagonists. L8429 which has a larger teriary butyl group in the para position proved to be neither a strong β-adrenoceptor antagonist nor cardioselective in its actions. However, if one examines the steric configuration of the tertiary butyl group, it becomes apparent that this is a bulky constituent which has little freedom of rotation. These properties could possibly impair its attachment to receptor sites. A n-butyl group would have more freedom of rotation and with the longer chain might provide a greater degree of cardioselectivity in its blocking action. L8429 was also tested for its ability to block ouabain-induced arrhythmias in guinea-pigs (Dohadwalla, Freedberg & Vaughan Williams, 1969). Although some protection was seen at doses above 3 mg/kg, this compound's anti-arrhythmic properties were minimal.

There are probably other structural characteristics that can satisfy the requirements for proper receptor attachment for inducing cardioselective β-adrenoceptor blockade. Also if the present study was extended further, exceptions would no doubt be found to exist in the requirements reported herein. However, it is felt from the information gathered to date that the length of the substituent on the para position of the benzene ring may be one factor of importance in determining cardioselectivity of β-adrenoceptor antagonists. In the 3-amino-2 hydroxypropoxy anilide series (analogues of practolol) studied by Crowther, Howe & Smith (1971), adding a phenyl (compound 7) or a 4 chlorophenyl (compound 8) to the acetamide in the para position yielded compounds as potent as practolol on the heart, and with higher cardioselectivity, and further lengthening to benzyl (compound 9) increased cardiac potency without loss of cardioselectivity.

The development of more potent cardioselective blocking drugs presents several important therapeutic implications. These compounds are known to be less prone to precipitate asthmatic attacks and do not block the effects of isoprenaline or adrenaline on bronchial smooth muscle. Practolol, the only currently available cardioselective drug, has also been reported to be less liable than propranolol to induce congestive heart failure (Gent, Davis & McDonald, 1970). If practolol is indeed less of a myocardial depressant, it may be related to its cardioselective properties. Vaughan Williams *et al.* (1973) have suggested that patients with heart failure may be dependent on circulating levels of adrenaline to maintain open vascular channels for enhancement of venous return. Blockade of those peripheral β-adrenoceptors by a drug such as propranolol might lead to an elevated peripheral

resistance which in borderline cases might induce failure. Pressor responses to propranolol in animals have been reported by several investigators (Nakano & Kusakari, 1965; Kayaalp & Kiran, 1966; Yamamoto & Sekiya, 1972). Oxprenolol and sotalol have also been shown to provoke an increase in blood pressure (Regoli, 1970), while practolol is devoid of this effect. If cardioselectivity is an important factor in avoiding cardiac failure following β-adrenoceptor blockade, it would be useful to have drugs with a higher ratio of cardioselectivity than that exhibited by practolol.

The authors wish to express their appreciation to Mrs. Sue Morgan for her technical assistance and to Dr. Robert Duncan, Department of Biometry, Medical University of South Carolina for his assistance in analysing the data.

This work was supported in part by the following grants: HE10411, National Heart Institute, N.I.H., United Health and Medical Research Foundation of South Carolina and the Charleston Scientific and Cultural Foundation. We also gratefully acknowledge an equipment grant from the British Heart Foundation.

REFERENCES

ÅBLAD, B., BROGARD, M., CARLSSON, E. & EK, L. (1970). β-Adrenergic blocking properties of three allyl-substituted phenoxy propylamines. *Europ. J. Pharmac.*, **13**, 59–64.

COTTEN, M. DE V. & BAY, E. (1956). Direct measurement of changes in cardiac contractile force; relationship of such measurements to stroke work, isometric pressure gradient and other parameters of cardiac function. *Amer. J. Physiol.*, **187**, 122–134.

CROWTHER, A. F., HOWE, R. & SMITH, L. H. (1971). β-Adrenergic blocking drugs. X. 3-Amino-2 hydroxy propoxy-anilides. *J. Med. Chem.*, **14**, 511–513.

DOHADWALLA, A. N., FREEDBERG, A. S. & VAUGHAN WILLIAMS, E. M. (1969). Relevance of β-receptor blockade to ouabain-induced cardiac arrhythmias. *Br. J. Pharmac.*, **36**, 257–267.

GENT, G., DAVIS, T. C. & MCDONALD, A. (1970). Practolol in treatment of supraventricular cardiac dysrhythmias. *Brit. Med. J.*, **1**, 533–535.

KAYAALP, S. O. & KIRAN, B. K. (1966). Mechanism of a sympathomimetic action of propranolol in dog. *Br. J. Pharmac. Chemother.*, **28**, 15–22.

NAKANO, J. & KUSAKARI, T. (1965). Effect of propranolol on the peripheral circulation. *Proc. Soc. Exp. Biol. Med.*, **120**, 516–519.

REGOLI, D. (1970). Pressor action of beta blocking agents in rats. *Can. J. Physiol. Pharmacol.*, **48**, 481–489.

STEEL, R. G. O. & TOUIE, J. J. (1960). *Principles and Procedures of Statistics*, p. 107. N.Y.: McGraw-Hill Book Co., Inc.

VAUGHAN WILLIAMS, E. M. & PAPP, J. G. (1970). The effect of oxprenolol on cardiac intracellular potentials in relation to its anti-arrhythmic, local anaesthetic and other properties. *Postgrad. Med. J. Nov. Suppl.*, **22**, 32.

VAUGHAN WILLIAMS, E. M., BAGWELL, E. E. & SINGH, B. N. (1973). Cardiospecificity of β-receptor blockade. A comparison of the relative potencies on cardiac and peripheral vascular β-adrenoceptors of propranolol, of practolol and its ortho-substituted isomer, and of oxprenolol and its para-substituted isomer. *Cardiovasc. Res.* **7**, 226–240.

YAMAMOTO, J. & SEKIYA, A. (1972). Further studies on the pressor action of propranolol in the rat. *Arch. int. Pharmacodyn.*, **198**, 347–354.

(*Received December* 23, 1972)