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Relaxant and β_2-adrenoceptor blocking activities of (±)-, (+)- and (−)-pindolol on the rat isolated aorta

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Abstract—The KCl contracted rat aorta is relaxed by procaterol, (±)-, (+)- and (−)-pindolol. The relaxations to procaterol, but not to (±)-pindolol, were prevented by ICI 118,551 at 10^{-6} M. The relaxations to (±)-pindolol are, therefore, not due to β-adrenoceptor agonism. At 10^{-7} M ICI 118,551, (±)-, (+)- and (−)-pindolol were β_2-adrenoceptor antagonists as they inhibited the relaxant responses of the aorta to procaterol.

(±)-Pindolol is classified as a β-adrenoceptor antagonist which also exerts partial agonism at β-adrenoceptors (Fitzgerald 1984). β-Adrenoceptor antagonists with partial agonism have the potential to cause both vasodilation and vasoconstriction by acting as agonists and antagonists at β-adrenoceptors, respectively. The vasodilator and vasoconstrictor activity of (+)- and (−)-pindolol have not been determined. The present study has examined the effects of (±)-, (+)- and (−)-pindolol on the rat isolated aorta and their ability to cause a small relaxation of the KCl contracted preparation. The effects of ICI 118,551 (a β-adrenoceptor antagonist) on these relaxations were also examined. Finally, the effects of (±)-, (+)- and (−)-pindolol on the relaxant responses of the rat aorta to procaterol (a potent and selective β_2-adrenoceptor agonist, O'Donnell & Wanstall 1985) were investigated.

Materials and methods

Male Wistar rats (250–350 g) were stunned and exsanguinated. The thoracic aorta was removed and placed in Krebs solution. All experiments were performed in the presence of a modified Krebs solution [composition (mM): NaCl 116, KCl 5·4, $CaCl_2$ 2·5, $MgCl_2$ 1·2, $NaHPO_4$ 1·2, $NaHCO_3$ 22·0, D-glucose 11·2, Na_2 EDTA 0·04] which was bubbled with 5% CO_2 in oxygen at 37° C. Contractile responses were measured isometrically with force displacement transducers (Grass model FTO 3C) and displayed on a polygraph (Grass model 79B). In each series of experiments, the groups of individual values obtained were compared by Student's paired *t*-test and were considered to be significantly different when $P<0·05$. Mean values ± s.e.m. were also obtained.

A ring of rat aorta was suspended between stainless steel hooks under 1 g tension in 5 mL organ baths containing Krebs solution. Tissues were equilibrated for 30 min before exposure to phenoxybenzamine at 5×10^{-5} M for 30 min to block α-adrenoceptors and extraneuronal uptake. Tissues were washed for 20 min and then contracted by ≥ 150 mg by the addition of KCl to the organ bath to give a final concentration of $2–3\times10^{-2}$ M. When the contraction was constant, three series of experiments were performed.

The effects of pindolol alone compared with those of procaterol. A cumulative challenge with procaterol, (±)-, (+)- or (−)-pindolol at $10^{-9}/10^{-8}$–$10^{-5}/10^{-4}$ M was made on a 4 min cycle.

The effects of ICI 118,551 on the relaxant responses to procaterol and pindolol. A 10 min challenge to procaterol or (±)-pindolol at 10^{-6} M was made. Tissues were then washed for 60 min in the absence or presence of ICI 118,551 at 10^{-6} M before recontracting the rat aorta with KCl to a constant level and then repeating the challenge to procaterol or pindolol.

The effects of ICI 118,551 and pindolol on procaterol response curves. Three successive challenges of the KCl contracted aorta with procaterol produced identical relaxant curves (Doggrell 1988). Each aorta was initially given a cumulative 4 min cycle challenge with procaterol in the absence of drugs. Tissues were then equilibrated for 45 min in the presence of ICI 118,551 or pindolol before the procaterol challenge was repeated on the recontracted aorta. Tissues were then equilibrated for a further 45 min in the presence of a higher concentration of drug being tested, before a third contraction with KCl and challenge with procaterol.

Assessment of data. The maximal decrease in contractile response to each concentration of procaterol or pindolol was measured. These relaxant responses were calculated as a percentage of the KCl contraction. When successive procaterol relaxant curves were obtained, if the maximal relaxant responses to procaterol expressed as a percentage of the KCl contraction were not significantly different between curves, all the relaxant responses were calculated as a percentage of the maximal relaxant response to procaterol i.e. normalized. For each normalized concentration-response curve, a slope and pD_2 value was determined. The slope of the procaterol response curve (difference in percentage maximum of the response/unit of logarithm molar concentration of procaterol) and pD_2 value were computed by regression line analysis over the steepest part of the response curve, which was usually between 20–80% of the maximum response. For each tissue, the ability of the drug to alter responses to an agonist was expressed as the concentration ratio at 50% relaxation (the antilogarithm of the difference between the pD_2 values in the presence and absence of the drug). When the effects of a drug were apparently compatible with competitive antagonism (i.e. there was no significant difference between the slopes of the response curves but a significant reduction in the pD_2 values obtained after the addition of antagonist) pA_2 values were determined. For each tissue a pA_2 value was calculated from the formula $pA_2=pA_x+\log(x-1)$ where pA_x is the negative logarithm of the molar concentration of drug and x is the agonist concentration ratio at 50% relaxation.

Drugs used. The drugs used were all donated and were ICI 118,551 [erythro-(±)-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol; ICI Ltd], (±)-, (+)- and (−)-pindolol (Sandoz) dissolved in equimolar tartaric acid and procaterol hydrochloride (Warner Lambert).

Results

The effects of drugs alone (Fig. 1). When the aorta had been contracted by the addition of KCl, it was relaxed by procaterol, ≥ 10^{-9} M. (±)-, (+)- and (−)-pindolol, ≥ 10^{-7} M, all relaxed the aorta to a similar extent; the relaxations were smaller than those to procaterol ($P\geq0·05$ for each concentration).

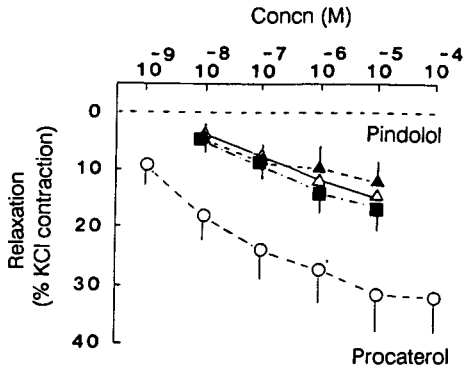

FIG. 1. Relaxant responses of the KCl contracted rat aorta. Responses to procaterol (○), (±)-pindolol (△), (+)-pindolol (▲) and (−)-pindolol (■). All relaxations are calculated as a percentage of the KCl contraction. Each value is the mean ± s.e.m. from 8 to 12 animals.

The effects of ICI 118,551 on the relaxant responses. Two successive 10 min challenges of the KCl contracted aorta to procaterol or (±)-pindolol (both at 10^{-6} M) produced relaxations that were not significantly different (n = 8, data not shown). The addition of ICI 118,551 at 10^{-6} M between challenges significantly reduced the relaxations to procaterol from 38% of the KCl contraction ± 8 (s.e.m.) to 19% ± 3 (n = 8, $P \geq 0.05$). The relaxant responses to (±)-pindolol were not altered by ICI 118,551 being 20% of the KCl contraction ± 4 before and 25% ± 3 (n = 8) after treatment with ICI 118,551.

The effects of ICI 118,551 and pindolol on procaterol response curves. ICI 118,551 (3×10^{-8} and 10^{-7} M), (±)-, (+)- and (−)-pindolol (all at 10^{-8}–10^{-7} M) produced rightward displacements of the procaterol relaxant response curves with no reduction in the maximum response (Figs 2, 3). The submaximal responses to procaterol were inhibited by ICI 118,551 at 3×10^{-8} and 10^{-7} M, by (±)-pindolol at 10^{-8} and 10^{-7} M, by (+)-pindolol at 10^{-8} and 10^{-7} M and by (−)-pindolol at 10^{-8} and 10^{-7} M, (see Table 1 for pD_2 values). Analysis of the slopes at the steepest part of the individual curves for procaterol illustrated that the slopes were not significantly different in the absence and presence of ICI 118,551 or (±)-, (+)- and (−)-pindolol and are therefore considered parallel (Table 1). In some cases the mean response curves appeared to be more shallow in the presence of these antagonists (Figs 2, 3). ICI 118,551 (mean $pA_2 = 8.9$), (±)-pindolol ($pA_2 = 8.7$) and (−)-pindolol ($pA_2 = 8.6$) were equipotent as β_2-adrenoceptor antagonists. (+)-Pindolol ($pA_2 = 9.3$) was significantly more potent than (±)- or (−)-pindolol as a β_2-adrenoceptor antagonist ($P \leq 0.05$).

(±)-Pindolol decreases total peripheral resistance in man (Atterhög et al 1976; Velasco et al 1980). A study of the effect of (+)- and (−)-pindolol on the dog isolated mesenteric artery has shown that (+)- and (−)-pindolol are equieffective vasodilators (Clark & Bertholet 1983). The present study, using the rat aorta, confirms that (±)-, (+)- and (−)-pindolol are equipotent vasorelaxants.

It has been suggested that the vasodilation observed with (±)-pindolol may be due to agonism at β_2-adrenoceptors (Fitzgerald 1984). However, Clark & Bertholet (1983) noted that the evidence for this was incomplete and inconclusive. Thus although propranolol, a nonselective β-adrenoceptor antagonist, did inhibit the vasodilator action of pindolol in the dog mesenteric artery, the concentration required to produce a measurable displacement of the dose-response curve for pindolol (i.e. 10^{-7} M propranolol) produced a much greater inhibition of the responses to isoprenaline. ICI 118,551 is a selective β_2-adrenoceptor antagonist having a pA_2 of 8.7 at β_2- (guinea-pig trachea) and 7.0 at β_1-adrenoceptors (guinea-pig atria, O'Donnell & Wanstall 1980). The rat aorta contains both β_1- and β_2-adrenoceptors mediating relaxation with the β_1-adrenoceptors being the minor population (O'Donnell & Wanstall 1984). Procaterol is a highly selective β_2-adrenoceptor agonist which relaxes the KCl-contracted rat aorta (O'Donnell & Wanstall 1985, and this study). The present study illustrates that ICI 118,551 inhibits the procaterol response curves of the rat aorta with a pA_2 of 8.9. However, the relaxation to (±)-pindolol was not prevented by pretreatment with ICI 118,551 at 10^{-6} M, a concentration that would produce antagonism at β_1- and β_2-adrenoceptors. Consequently it is unlikely that these relaxations

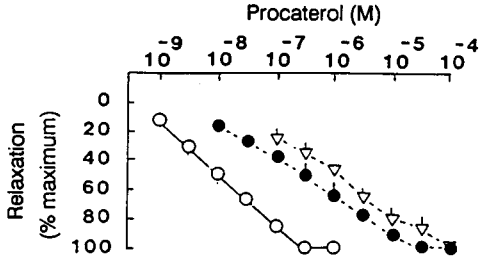

FIG. 2. Effect of ICI 118,551 on procaterol response curves. Responses in the absence (○) and presence of ICI 118,551 at 3×10^{-8} (●) and 10^{-7} M (▽). Responses are calculated as a percentage of the maximum relaxation and plotted against the log of the molar concentration of procaterol. Each value is the mean ± s.e.m. from 9 preparations.

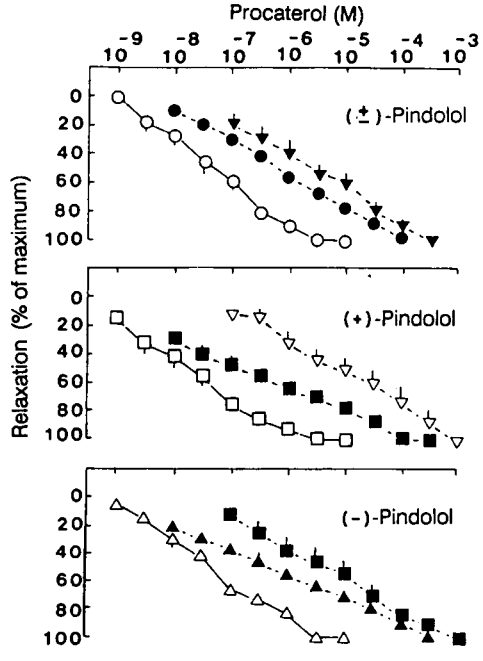

FIG. 3. Effect of (±)- (top), (+)- (middle), and (−)- (bottom) pindolol on procaterol response curves. Top: responses in the absence (○) and presence of (±)-pindolol at 10^{-8} (●) and 10^{-7} M (▼). Middle: responses in the absence (□) and presence of (+)-pindolol at 10^{-8} (■) and 10^{-7}M (▽). Bottom: responses in the absence (△) and presence of (−)-pindolol at 10^{-8} M (▲) and 10^{-7} M (■). Responses are calculated as a percentage of the maximum relaxation and plotted against the log of the molar concentration of procaterol. Each value is the mean ± s.e.m. from 8 to 9 animals.

Table 1. Effects of procaterol response curves.

Antagonist concn (M)	Slopes[a]	pD_2[a]	pA_2[a]
Control	47±8 (9)	7·90±0·12 (9)	
ICI 118,551, 3×10^{-8}	35±7 (9)	6·56±0·17 (9)*	8·9±0·2 (9)
ICI 118,551, 10^{-7}	37±7 (9)	5·99±0·13 (9)*	8·9±0·2 (9)
Control	56±7 (9)	7·37±0·16 (9)	
(±)-Pindolol, 10^{-8}	46±7 (9)	6·34±0·23 (9)*	8·9±0·2 (9)
(±)-Pindolol, 10^{-7}	45±10 (9)	5·87±0·28 (9)*	8·5±0·3 (9)
Control	43±8 (8)	7·85±0·20 (8)	
(+)-Pindolol, 10^{-8}	33±7 (8)	6·59±0·24 (8)*	9·1±0·5 (8)
(+)-Pindolol, 10^{-7}	53±10 (8)	5·18±0·25 (8)*	9·5±0·2 (8)
Control	50±7 (8)	7·32±0·09 (8)	
(−)-Pindolol, 10^{-8}	38±8 (8)	6·39±0·26 (8)*	8·5±0·5 (8)
(−)-Pindolol, 10^{-7}	51±9 (8)	5·51±0·35 (8)*	8·7±0·4 (8)

[a]Mean±s.e.m.
(n) = number of animals.
* $P<0.05$, paired t-test with own control.

are due to stimulation of either the β_1- or β_2-adrenoceptors of the rat aorta.

Adrenaline, and possibly noradrenaline, stimulates vascular β_2-adrenoceptors to produce vasodilation (Fitzgerald 1984). Inhibition of this vasodilation produces an increase in blood pressure (Fitzgerald 1984) and is an unwanted effect in hypertension. Previous studies using both the guinea-pig trachea and the soleus muscle (Jeppsson et al 1984; Walter et al 1984) have demonstrated that (−)-, but not (+)-pindolol is a potent β_2-adrenoceptor antagonist. In the present study using the rat aorta, (−)-pindolol had a similar potency as a β_2-adrenoceptor antagonist as previously reported using the guinea-pig trachea/soleus muscle. In this study (+)-pindolol was also a potent β_2-adrenoceptor antagonist. Recently I showed KF-4317 (4-(2-hydroxy-3-((1-methyl-3-phenylpropyl)amino)propoxy)benzeneacetamide), which is a weak antagonist at the β_2-adrenoceptors of the guinea-pig trachea (Kubo et al 1983), to be a potent antagonist at the β_2-adrenoceptors of the rat aorta (Doggrell 1988). This indicates that there may be some differences between the β_2-adrenoceptors of the guinea-pig trachea and aorta.

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References

Atterhög, J.-H., Duner, H., Pernow, M. D. (1976) Experience with pindolol, a beta-receptor blocker, in the treatment of hypertension. Am. J. Medicine 60: 872–876

Clark, B. J., Bertholet, A. (1983) Effects of pindolol on vascular smooth muscle. Gen. Pharmacol. 14: 117–119

Doggrell, S. A. (1988) Relaxant and β_2-adrenoceptor blocking activities of labetalol, dilevalol, amosulalol and KF-4317 on the rat isolated aorta. J. Pharm. Pharmacol. 40: 812–815

Fitzgerald, J. D. (1984) β-Adrenoceptor blocking drugs. In: van Zwieten, P. A. (ed.) Pharmacology of Antihypertensive Drugs. Elsevier, Amsterdam, New York and Oxford, pp 249–306

Jeppsson, A.-B., Johansson, V., Walbeck, B. (1984) Steric aspects of agonism and antagonism at β-adrenoceptors: experiments with the enantiomers of terbutaline and pindolol. Acta Pharmacol. Toxicol. 54: 285–291

Kubo, K., Nakamura, J., Karasawa, A., Shuto, K., Nakamizo, N. (1983) Pharmacology of 4-(2-hydroxy-3-[(1-methyl-3-phenyl-propyl)-amino] propoxy) benzeneacetamide (KF-4317). Drug Res. 33: 926–931

O'Donnell, S. R., Wanstall, J. C. (1980) Evidence that ICI 118,551 is a potent, highly beta$_2$-selective adrenoceptor antagonist and can be used to characterise beta-adrenoceptor populations in tissues. Life Sci. 27: 671–677

O'Donnell, S. R., Wanstall, J. C. (1984) Beta-1 and beta-2 adrenoceptor-mediated responses in preparations of pulmonary artery and aorta from young and aged rats. J. Pharmacol. Exp. Ther. 228: 733–738

O'Donnell, S. R., Wanstall, J. C. (1985) Responses to the β_2-selective agonist procaterol of vascular and atrial preparations with different functional β-adrenoceptor populations. Br. J. Pharmacol. 84: 227–235

Velasco, M., Urbina-Quintana, A., Morillo, J., Guevara, J., Ramirez, A., Hernandez-Pieretti, O. (1980) Cardiac and systemic hemodynamic effects of pindolol in hypertensive patients. Curr. Ther. Res. 28: 972–978

Walter, M., Lemoine, H., Kaumann, A. J. (1984) Stimulant and blocking effects of optimal isomers of pindolol on the sinoatrial node and trachea of guinea pig. Role of β-adrenoceptor subtypes in the dissociation between blockade and stimulation. Naunyn-Schmiedeberg's Arch. Pharmacol. 327: 159–175