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Effect of atenolol and pindolol on the phorbol ester-induced coronary vasoconstriction in the isolated perfused heart of the rat

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1 The effects of atenolol (β_1-adrenoceptor antagonist without partial agonistic activity) and pindolol (β_1- and β_2-antagonist with partial agonistic activity) were studied on basal coronary vascular tone and on the phorbol ester-induced coronary vasoconstriction in the rat perfused heart.

2 The addition of the phorbol ester 12-0-tetradecanoyl-phorbol-13-acetate (TPA; 1.8×10^{-8}–1.6×10^{-7} M) into the perfusion fluid during perfusion of rat heart at constant flow caused a dose-dependent, sustained increase in perfusion pressure. The vasoconstrictor response in hearts of reserpine-treated rats to infusion of TPA was similar to that of non-reserpine treated hearts.

3 Infusion of a calcium channel agonist Bay K 8644 at a concentration of 4×10^{-7} M enhanced, whereas isoprenaline (1×10^{-5} M), dibuturyl-cyclic AMP (1.6×10^{-4} M) and forskolin (1×10^{-6} M), which elevate intracellular concentrations of cyclic AMP, all inhibited the coronary vasoconstriction induced by TPA.

4 Pindolol, in doses which produced comparable inhibition of isoprenaline-induced tachycardia, dose-dependently attenuated the phorbol ester-induced increase in perfusion pressure, whereas atenolol had no effect. The inhibitory action of pindolol (2×10^{-5} M) on TPA-induced vasoconstriction was blocked by addition of 2.2×10^{-5} M propranolol into the perfusion fluid. When infused alone, atenolol (2×10^{-4} M) significantly increased coronary vascular tone, but pindolol had no effect.

5 The present results indicate that pindolol has coronary vasodilator properties due to stimulation of vascular β-adrenoceptors. If stenosis dilatation of coronary artery spasm is an important component of the anti-anginal effect of β-blocking drugs, the possession of partial agonistic property by a β-blocking drug may be of importance in maintaining coronary flow.

Introduction

Coronary blood flow is determined by several factors including perfusion pressure, coronary vascular resistance, cardiac metabolic demands and vasomotor tone (Feigl, 1983). The heart and coronary circulation receive a plentiful sympathetic nerve supply and numerous studies have established the presence of both α- and β-adrenoceptors in animal and human coronary arteries (Klocke *et al.*, 1965; Parratt, 1965; Feigl, 1967; Pitt *et al.*, 1967; Mudge *et al.*, 1976). An increase in α-adrenoceptor-mediated tone has been associated with coronary artery vasoconstriction, whereas α-adrenoceptor blockade has been shown to reduce attacks of variant angina pectoris and abolish coronary vasoconstriction (Yasue *et al.*, 1979a). Increases in β-adrenoceptor-mediated tone have been associated with coronary artery vasodilatation (Vatner *et al.*, 1982), whereas coronary artery vasoconstriction and increases in coronary vascular resistance can be induced by sympathetic stimulation after β-adrenoceptor blockade (Pitt *et al.*, 1967; Yasue *et al.*, 1976; Kern *et al.*, 1983; Vatner & Hintze, 1983). In patients with variant angina, exacerbation of coronary spasm after β-adrenoceptor blockade has been described (Yasue *et al.*, 1979a,b; Robertson *et al.*, 1982).

Several β-blocking drugs appear to stimulate partially, in addition to blocking, the β-adrenoceptor (Frishman, 1987). A β-blocking drug with a significant degree of partial agonistic or intrinsic sympathomimetic activity causes a smaller decrease in heart rate and cardiac output than ones without (Frishman, 1982; Taylor *et al.*, 1982), and may

produce a lesser decrease in peripheral blood flow (for recent reviews, see Frishman, 1987; Prichard, 1987). Previously, tumour promoting phorbol esters such as 12-0-tetradecanoyl-phorbol-13-acetate (TPA), known activators of protein kinase C, have been shown to elicit sustained contraction in several vascular smooth muscle preparations (Rasmussen *et al.*, 1984; Danthuluri & Deth, 1984; Forder *et al.*, 1985; Ruskoaho *et al.*; 1985; Spedding, 1986). The present experiments were designed to study the effect of β_1-adrenoceptor blockers without agonistic activity (atenolol) and β_1- and β_2-adrenoceptor blockers with high partial agonistic activity (pindolol) on baseline coronary vascular tone and on the phorbol ester-induced coronary vasoconstriction in the rat perfused heart, in doses designed to produce comparable β_1-blockade.

Methods

Perfused hearts

The rat isolated perfused heart preparation used in this study was similar to that previously described (Ruskoaho *et al.*, 1985). Male Wistar rats (weighing 250–350 g) were maintained on rat chow *ad libitum* in rooms with a 12 h light and 12 h dark cycle. The blood of the rats was anticoagulated with heparin (500 u kg^{-1} body weight, i.p.) and they were decapitated 20 min later. The abdominal cavity was immediately opened, the diaphragm transected, and lateral incisions were made along both sides of the rib cage. The anterior chest wall was retracted and the heart was cooled with perfusion fluid (4–10°C). The aorta was cannulated superior to the aortic valve and retrograde perfusion was begun with a modified Krebs-Henseleit bicarbonate buffer, pH 7.40, equilibrated with 95% O_2/5% CO_2, at 37°C. Final concentrations of the salts in the buffer (mmol l^{-1}) were: NaCl 113.8, $NaHCO_3$ 22.0, KCl 4.7, KH_2PO_4 1.2, $MgSO_4 \cdot 7H_2O$ 1.1, $CaCl_2 \cdot 2H_2O$ 2.5 and glucose 11.

Variations in perfusion pressure arising from changes in coronary vascular resistance were recorded on a Grass polygraph (model 7DA) with a pressure transducer (Micron, MP-15) situated on a side-arm of the aortic cannula. Isometric force of contraction was recorded by a strain gauge transducer (Grass FTO3) connected to the Grass polygraph. The output was damped to give a mean contractile force. Heart rate was counted from contractions by the Grass tachograph. The hearts were submitted to a resting tension of 2 g. During the equilibration period (60 min) the hearts were perfused with a peristaltic pump (Ismatec) using a flow rate of 10 ml min^{-1} and then switched to constant flow of 5 ml min^{-1}.

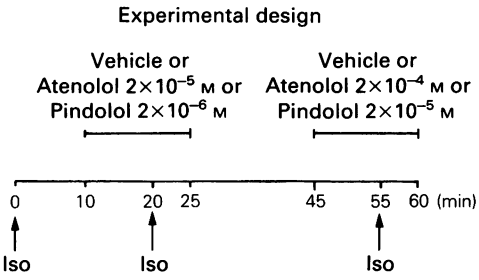

Figure 1 Experimental protocol. Iso = isoprenaline.

Experimental design

In order to demonstrate the β-antagonistic effects of atenolol and pindolol at the concentrations used, a series of experiments were performed in which the responses to bolus injections of isoprenaline (2 μg), given in a volume of 100 μl into the aortic perfusion cannula just above the heart, were studied before and during perfusion with antagonists. The protocol is illustrated in Figure 1. Perfusion time with the antagonist was 15 min; each preparation was tested at two concentrations of antagonist. Antagonist (or vehicle) were added as a continuous infusion via an infusion pump (Braun) using an infusion rate of 0.5 ml min^{-1}. The final perfusion cannula concentrations of all agents are given in this paper.

In the second series of experiments, vehicle or drugs alone or in combination with TPA were added into the aortic perfusion cannula as a continuous infusion via an infusion pump using a rate of 0.5 ml min^{-1} for 30 min, after a 10 min control period. Control experiments were run with the solvents used: dimethylsulphoxide (TPA), ethanol (forskolin and Bay K 8644), tartaric acid (pindolol) and Krebs-Henseleit buffer. Addition of an appropriate concentration of each caused no significant change in perfusion pressure.

To study the effect of depletion of endogenous noradrenaline stores on the responses to the phorbol ester, rats were treated with reserpine (5 mg kg^{-1} i.p.) 48 and 24 h before the initiation of the perfusion experiment. This treatment inhibits the tyramine-induced increase in heart rate in the pithed rat (Savola *et al.*, 1988).

Finally, the coronary vascular responses to bolus injections of isoprenaline, atenolol and pindolol were studied. In these experiments, during the equilibration period the hearts were perfused using the flow rate of 10 ml min^{-1} as described above. To increase basal perfusion pressure above 100 mmHg,

Table 1 Effect of infusions of atenolol and pindolol on heart rate (beats min^{-1}) in the rat perfused heart

Treatment	*Time* (min)								
	−10	*−5*	*0*	*+5*	*+10*	*+15*	*+20*	*+25*	*+30*
Vehicle	248 ± 18	249 ± 17	249 ± 17	245 ± 15	247 ± 17	249 ± 17	250 ± 17	248 ± 16	253 ± 16
Atenolol 2×10^{-5} M	254 ± 23	262 ± 24	268 ± 26	271 ± 18	274 ± 16	269 ± 16	266 ± 18	263 ± 18	263 ± 16
Atenolol 2×10^{-4} M	281 ± 20	280 ± 20	281 ± 20	276 ± 19	273 ± 19	270 ± 22	266 ± 22	264 ± 22	263 ± 21
Pindolol 2×10^{-6} M	282 ± 23	279 ± 23	277 ± 23	296 ± 25	300 ± 28	302 ± 27	304 ± 27	303 ± 27	303 ± 26
Pindolol 2×10^{-5} M	284 ± 26	282 ± 26	281 ± 25	287 ± 22	282 ± 24	279 ± 22	278 ± 22	276 ± 20	275 ± 20

After a 10 min control period, atenolol and pindolol (time: 0 min) were added into the perfusion fluid for 30 min. Results are expressed as means ± s.e. mean of 5–6 results obtained from different isolated hearts.

the perfusion rate was then switched to constant flow of 16–22 ml min^{-1}. After stabilization of perfusion pressure (10–20 min), vehicle, isoprenaline (2 μg), atenolol (200 μg) or pindolol (100 μg), each given in a volume of 100 μl, were injected into the aortic perfusion cannula at intervals of at least 10 min and changes in perfusion pressure were ascertained. Each heart received vehicle and one drug injection. In separate experiments, the effect of bolus injections of pindolol (200 μg 100 μl^{-1}) against coronary vasoconstriction produced by lysine^8- vasopressin (5 u ml^{-1} perfusate) were studied.

Materials

Drugs used in this study were: atenolol hydrochloride (Star Pharmaceutical Co., Tampere, Finland), pindolol (Sandoz Ltd., Basel, Switzerland), propranolol hydrochloride (Orion Pharmaceutical Co., Espoo, Finland), isoprenaline hydrochloride (Sigma Chemical Co., St. Louis, U.S.A.), Bay K 8644 (Bayer, Leverkusen, F.R.G.), lysine^8-vasopressin (Sandoz Ltd., Basel, Switzerland), the phorbol ester, 12-0-tetradecanoyl-phorbol-13-acetate (TPA), forskolin and dibuturyl-cyclic AMP (dibuturyl- adenosine 3′:5′-cyclic monophosphate; Sigma Chemical Co.), and heparin (Medica, Helsinki, Finland). Other chemicals not mentioned were from Sigma. TPA was dissolved in DMSO, Bay K 8644 and forskolin in ethanol, and pindolol in tartaric acid. Final concentration of each solvent was less than 0.03%. All other drugs tested were dissolved in Krebs-Henseleit solution.

Analysis of data

The results are expressed as means ± s.e. mean. The data were analysed with two- or one-way analysis of variance (ANOVA). For the comparison of statistical significance between two dependent groups Student's

Table 2 Effect of infusions of atenolol and pindolol on the contractile force (g) in the rat perfused heart

Treatment	*Time* (min)								
	−10	*−5*	*0*	*+5*	*+10*	*+15*	*+20*	*+25*	*+30*
Vehicle	2.03 ± 0.02	1.98 ± 0.02	1.98 ± 0.02	2.03 ± 0.03	2.01 ± 0.03	2.04 ± 0.03	2.04 ± 0.03	2.01 ± 0.04	2.00 ± 0.04
Atenolol 2×10^{-5} M	1.84 ± 0.05	1.81 ± 0.04	1.81 ± 0.05	1.76 ± 0.09	1.76 ± 0.09	1.74 ± 0.10	1.74 ± 0.11	1.74 ± 0.12	1.76 ± 0.13
Atenolol 2×10^{-4} M	1.97 ± 0.03	1.93 ± 0.03	1.91 ± 0.03	1.99 ± 0.09	2.03 ± 0.08	2.07 ± 0.010	1.92 ± 0.08	1.87 ± 0.09	1.89 ± 0.15
Pindolol 2×10^{-6} M	2.01 ± 0.02	2.00 ± 0.03	1.92 ± 0.04	2.00 ± 0.04	1.95 ± 0.05	1.94 ± 0.04	1.90 ± 0.04	1.86 ± 0.04	1.85 ± 0.05
Pindolol 2×10^{-5} M	1.98 ± 0.03	1.96 ± 0.04	1.98 ± 0.03	1.95 ± 0.03	1.93 ± 0.03	1.91 ± 0.04	1.89 ± 0.04	1.87 ± 0.04	1.87 ± 0.04

Results are expressed as means ± s.e. mean of 5–6 rats. For other details see legend to Table 1.

Table 3 Effect of infusions of atenolol and pindolol on perfusion pressure (mmHg) in the rat perfused heart

Treatment	*Time* (min)								
	−10	*−5*	*0*	*+5*	*+10*	*+15*	*+20*	*+25*	*+30*
Vehicle	23 ± 2	24 ± 2	24 ± 2	26 ± 2	27 ± 2	26 ± 2	27 ± 2	26 ± 2	27 ± 2
Atenolol 2×10^{-5} M	28 +2	28 ± 2	28 ± 2	30 ± 2	31 ± 3	31 ± 2	31 ± 2	32 ± 2	32 ± 3
Atenolol 2×10^{-4} M	33 ± 4	33 ± 4	33 ± 4	39 ± 4	44 ± 5	61 ± 11	87 ± 17	116 ± 22	126 ± 23
Pindolol 2×10^{-6} M	27 ± 3	27 ± 3	27 ± 3	29 ± 3	30 ± 4	31 ± 4	31 ± 4	31 ± 4	32 ± 4
Pindolol 2×10^{-5} M	31 ± 3	31 ± 3	31 ± 4	34 ± 5	35 ± 5	36 ± 6	37 ± 6	39 ± 7	39 ± 8

Results are expressed as means ± s.e. mean of 5–6 rats. For other details see legend to Table 1.

t test for paired data was used. For the multiple comparison one-way analysis of variance followed by the Bonferroni t test was used. Differences at the 95% level were considered statistically significant.

Results

Effects of atenolol and pindolol on heart rate, contractile force and perfusion pressure

Mean initial perfusion pressure was 33.5 ± 1.4 mmHg, mean initial heart rate was 260 ± 5 beats min^{-1} and mean initial contractile force was 1.9 ± 0.01 g ($n = 77$) in our rat heart preparation. There were few changes in heart rate or contractile force during the infusion of atenolol and pindolol into the coronary circulation. Addition of 2×10^{-4} M atenolol for 30 min decreased heart rate ($F = 7.1$, $P < 0.001$ between atenolol and vehicle), whereas the lower dose of pindolol (2×10^{-6} M) increased heart rate slightly ($F = 7.2$, $P < 0.001$) (Table 1). Infusion of pindolol decreased contractile force slightly (2×10^{-6} M: $F = 3.9$, $P < 0.001$; and 2×10^{-5} M: $F = 2.1$, $P < 0.04$ for drug and time interaction), whereas atenolol had no effect on contractile force (Table 2). The perfusion pressure increased markedly during infusion of the higher dose of atenolol ($F = 19.2$, $P < 0.001$), but pindolol treatment did not change the perfusion pressure significantly (2×10^{-5}: $F = 2.8$, $P < 0.08$) (Table 3).

Antagonism of isoprenaline-induced haemodynamic changes by atenolol and pindolol

Three consecutive injections of isoprenaline resulted in consistent increases in heart rate (Figure 2). Infusions of the β-adrenoceptor blocking drugs atenolol and pindolol, starting 10 min before the isoprenaline challenge (Figure 1), dose-dependently inhibited the isoprenaline-induced tachycardia, pindolol being about 10 times more potent than atenolol (Figure 2). Similarly, both drugs inhibited the increase of contractile force produced by isoprenaline (Table 4). Pindolol also inhibited isoprenaline-induced vasodilatation, whereas atenolol had only a limited effect (Table 4).

Effect of TPA on perfusion pressure

Addition of TPA into the perfusion fluid during constant flow perfusion of the rat heart caused a gradual sustained contraction which was dose-dependent (Figure 3). The concentration of 1.6×10^{-7} M TPA was selected for further experiments; compared to the vehicle, the perfusion pressure of isolated hearts treated with this dose of TPA was significantly higher 3 min after starting the infusion (50 ± 6 mmHg vs. 30 ± 3 mmHg, $P < 0.01$). Table 5 shows the baseline values and changes from baseline, either in actual values or % change, for perfusion pressure after each drug infusion.

Effect of Bay K 8644, forskolin and dibuturyl-cyclic AMP on TPA-induced coronary vasoconstriction

Infusion of a calcium channel agonist Bay K 8644 at a concentration of 4×10^{-7} M increased heart rate by 37% (323 ± 14 vs. 237 ± 8 beats min^{-1}, $P < 0.001$), contractile force by 54% (3.10 ± 0.13 vs. 2.01 ± 0.05 g, $P < 0.001$) and perfusion pressure by 96% ($F = 34.3$, $P < 0.001$, Figure 3). If Bay K 8644 was added to the perfusate together with TPA, Bay K 8644 significantly enhanced the phorbol-ester induced increase in perfusion pressure ($F = 10.1$, $P < 0.002$, Figure 3).

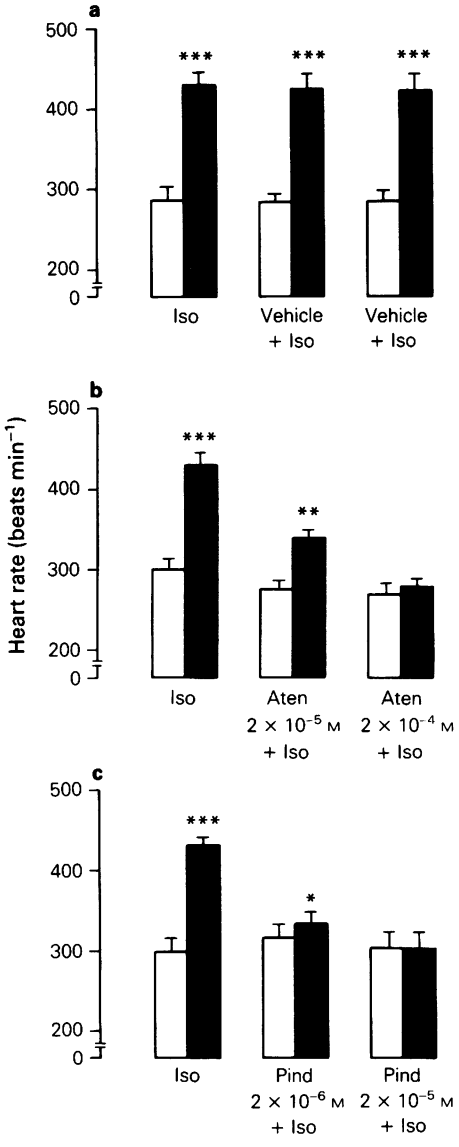

Figure 2 Effect of (b) atenolol (Aten) and (c) pindolol (Pind) on isoprenaline-induced increase in heart rate. The β-adrenoceptor blocking drugs were added as a continuous infusion using an infusion rate of 0.5 ml min^{-1} for 15 min; each preparation was tested at two concentrations of antagonist. The responses to bolus injections of isoprenaline (Iso) were studied 10 min after the infusion of antagonist (or (a) vehicle) was started. For details of protocol see Figure 1. The open columns represent control responses and the solid columns effect of test compounds. Results are expressed as means of 5–6 hearts; vertical lines indicate s.e. mean. *** Indicates a significance level $P < 0.001$; ** indicates $P < 0.01$; and * indicates $P < 0.05$ (Student's t test, paired).

Addition of 1×10^{-6} M forskolin into the perfusion fluid increased heart rate (331 ± 6 vs. 250 ± 7 beats min^{-1}, $P < 0.001$) and contractile force (2.59 ± 0.11 vs. 2.00 ± 0.02, $P < 0.001$), but did not significantly change the basal perfusion pressure ($F = 1.2$, $P < 0.32$ for drug and time interaction, Figure 3). When forskolin was added in combination with TPA, it effectively inhibited the phorbol ester-induced increase in coronary perfusion pressure ($F = 91.5$, $P < 0.001$, Figure 3). Similarly, infusion of a cyclic AMP derivative, dibuturyl-cyclic AMP, in combination with TPA, attenuated the phorbol ester-induced coronary vasoconstriction ($F = 10.4$, $P < 0.002$), but had no effect when infused alone ($F = 0.62$, $F < 1$) (Figure 3).

Effects of isoprenaline, atenolol and pindolol on TPA-induced vasoconstriction

Perfusion of the isolated heart with 1×10^{-5} M isoprenaline significantly increased heart rate (425 ± 8 vs. 273 ± 5 beats min^{-1}, $P < 0.001$) and contractile force (2.77 ± 0.09 vs. 1.86 ± 0.01 g, $P < 0.001$), but did not significantly change basal perfusion pressure over a 30 min period (Figure 4). If isoprenaline was added to the perfusate together with TPA, the phorbol ester-induced increase in perfusion pressure was significantly inhibited by treatment with isoprenaline ($F = 4.9$, $P < 0.02$).

Infusions of the β_1-receptor antagonist atenolol at concentrations which inhibited the isoprenaline-induced tachycardia were ineffective against the TPA-induced increase in perfusion pressure (2×10^{-5} M: $F = 0.5$ and 2×10^{-4} M: $F = 0.13$) (Figure 4). In contrast, pindolol caused a dose-dependent reduction of the phorbol ester-induced coronary vasoconstriction (2×10^{-6} M: $F = 1.9$, $P < 0.06$ and 2×10^{-5} M: $F = 6.5$, $P < 0.01$). When propranolol (2.2×10^{-5} M) was added to the perfusion fluid together with pindolol and TPA, the pressure increase produced by this combination did not differ from that induced by the phorbol ester alone ($F = 0.19$, $F < 1$) (Figure 4). When infused alone, propranolol (2.2×10^{-5} M) significantly inhibited the isoprenaline-induced increase in heart rate (heart rate: 37 ± 18 vs. 145 ± 12 beats min^{-1} in vehicle group, $P < 0.001$, $n = 4$) and contractile force (0.09 ± 0.03 vs. 0.93 ± 0.12 g, $P < 0.001$) and decrease in perfusion pressure (2 ± 1 vs. 99 ± 7 mmHg, $P < 0.001$).

Effect of reserpine pretreatment on TPA-induced coronary vasoconstriction

Addition of TPA into the perfusion fluid caused an increase in perfusion pressure ($F = 248.9$, $P < 0.001$)

Table 4 Effect of infusion of atenolol and pindolol on isoprenaline-induced increase in contractile force and decrease in perfusion pressure in the rat perfused heart.

Treatment	*Contractile force* (g) Control	Max.	%	*Perfusion pressure* (mmHg) Control	Max.	%
Iso	1.58	2.66***	+70	79	48*	−34
	± 0.07	± 0.10	± 10	± 10	± 5	± 4
Iso +	1.47	2.50***	+71	79	49*	−35
Aten 2×10^{-5} M	± 0.03	± 0.05	± 7	± 10	± 4	± 8
Iso +	1.50	2.10**	+41	76	61	−16
Pind 2×10^{-6} M	± 0.10	± 0.09	± 6	± 15	± 8	± 5
Iso	1.31	2.45***	+88	99	51***	−48
	± 0.05	± 0.06	± 5	± 7	± 5	± 4
Iso +	1.25	1.86**	+48	104	62	−35
Aten 2×10^{-4} M	± 0.03	± 0.10	± 8	± 21	± 6	± 8
Iso +	1.25	1.33	+6	97	96	−1
Pind 2×10^{-5} M	± 0.13	± 0.15	± 3	± 18	± 18	± 1

Atenolol (Aten) and pindolol (Pind) were added as a continuous infusion using an infusion rate of 0.5 ml min^{-1} for 15 min; each preparation was tested at two concentrations of antagonist. The responses to bolus injections of isoprenaline (Iso) were studied 10 min after the infusion of antagonist was started. For details of protocol see Figure 1. Results are expressed as means ± s.e. mean of 5–6 hearts. *** Indicates a significance level $P < 0.001$; ** indicates $P < 0.01$; and * indicates $P < 0.05$ (Student's t test, paired).

Table 5 Effect of various drugs on basal and phorbol ester-induced increase in perfusion pressure in rat isolated perfused hearts

Treatment	n	*Perfusion pressure* (mmHg) Control	Max.	Change (%)
Vehicle	6	24 ± 2	27 ± 2	13 ± 2
TPA 1.5×10^{-8} M	6	34 ± 5	96 ± 5***	201 ± 32
TPA 1.6×10^{-7} M	6	30 ± 3	116 ± 7***	303 ± 31
Bay K 8644 4×10^{-7} M	5	34 ± 1	67 ± 5**	21 ± 9
Bay K 8644 + TPA	6	30 ± 1	160 ± 11***	450 ± 60
Isoprenaline 1×10^{-5} M	6	29 ± 3	30 ± 4	1 ± 4
Isoprenaline + TPA	6	30 ± 1	63 ± 11**	112 ± 39
Db-cyclic AMP 1.6×10^{-4} M	5	28 ± 3	30 ± 3	2 ± 4
Db-cyclic AMP + TPA	5	30 ± 5	66 ± 16**	139 ± 77
Forskolin 1×10^{-6} M	6	39 ± 3	38 ± 2	−1 ± 4
Forskolin + TPA	6	38 ± 2	42 ± 2	9 ± 5
Atenolol 2×10^{-5} M	5	28 ± 2	31 ± 3	9 ± 4
Atenolol 2×10^{-5} M + TPA	5	31 ± 1	126 ± 12***	309 ± 46
Atenolol 2×10^{-4} M	5	33 ± 4	44 ± 5*	59 ± 178
Atenolol 2×10^{-4} M + TPA	5	27 ± 2	134 ± 13***	410 ± 44
Pindolol 2×10^{-6} M	5	27 ± 3	30 ± 4	11 ± 2
Pindolol 2×10^{-6} M + TPA	5	27 ± 3	105 ± 13***	267 ± 54
Pindolol 2×10^{-5} M	6	31 ± 4	35 ± 5	11 ± 4
Pindolol 2×10^{-5} M + TPA	6	22 ± 2	76 ± 16***	225 ± 50
Pindolol 2×10^{-5} M + Prop 2.2×10^{-5} M + TPA	6	29 ± 2	113 ± 14***	306 ± 36
Reserpine + TPA	6	32 ± 2	116 ± 11***	263 ± 22

After a 10 min control period, drugs alone or in combination with TPA (1.6×10^{-7} M) were added into the perfusion fluid for 30 min. Results are expressed as means ± s.e. mean. Control = perfusion pressure before infusion; max. = perfusion pressure 10 min after the infusion was started. TPA = 12-tetradecanoyl-phorbol-13-acetate, Prop = propranolol and Db-cyclic AMP = dibuturyl-cyclic AMP. Statistical significance was ascertained by using the one-way analysis of variance (ANOVA) followed by the Bonferroni t test as appropriate. *** Indicates a significance level $P < 0.001$; ** indicates $P < 0.01$; and * indicates $P < 0.05$.

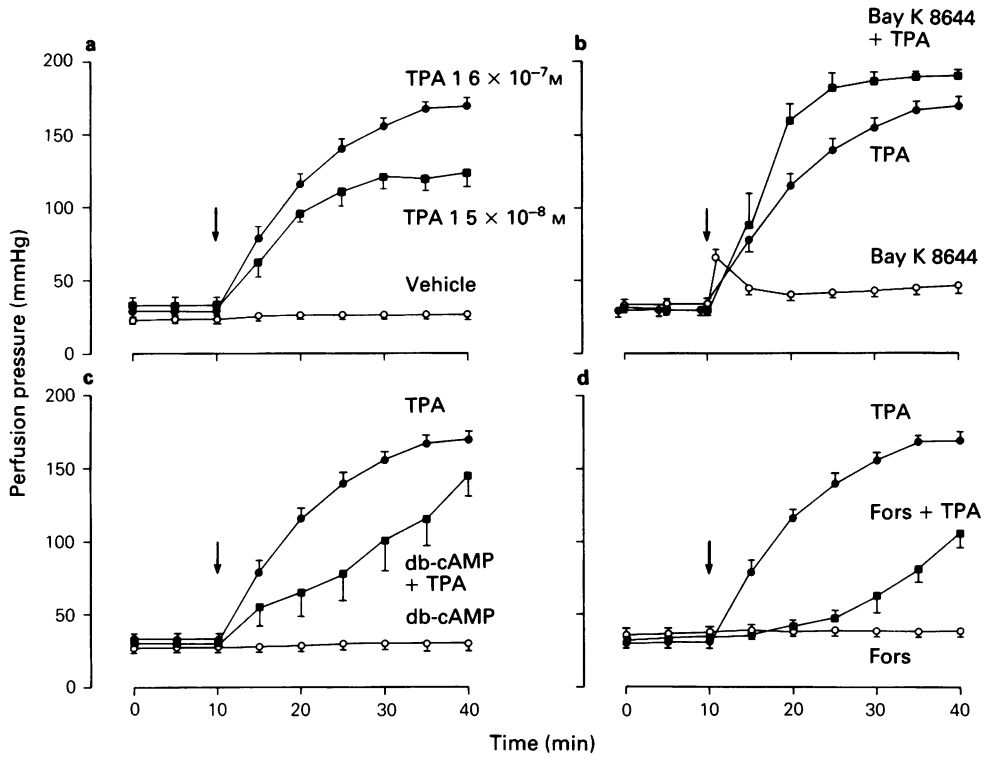

Figure 3 Effect of (a) 12-0-tetradecanoyl-phorbol-13-acetate (TPA), (b) Bay K 8644, (c) dibuturyl cyclic AMP and (d) forskolin on baseline and phorbol ester-induced increase in perfusion pressure in rat isolated perfused hearts. After a 10 min control period (arrows), drugs alone or in combination with TPA (1.6×10^{-7} M) were added to the perfusion fluid for 30 min. Each point is the mean value of from 5–6 separate experiments run on different isolated hearts; vertical lines indicate s.e. mean. db-cAMP = dibuturyl-cyclic AMP; Fors = forskolin.

in hearts of reserpine-treated rats. The coronary vasoconstrictor response to infusion of TPA at a concentration of 1.6×10^{-7} M was similar to that of hearts from control (not reserpine-treated) rats (Figure 4, Table 5).

Effect of bolus injections of isoprenaline, atenolol and pindolol on perfusion pressure

Before bolus injections of vehicle, isoprenaline (2 μg), atenolol (200 μg) and pindolol (100 μg), mean baseline perfusion pressures were 114 ± 4 mmHg ($n = 8$), 124 ± 8 mmHg ($n = 7$), 120 ± 5 mmHg ($n = 4$) and 131 ± 11 mmHg ($n = 5$), respectively. Vehicle injection had no significant effect on perfusion pressure (change: -1 ± 4 mmHg, $n = 12$), whereas both isoprenaline (-45 ± 7 mmHg, $n = 7$, $P < 0.001$ vs. vehicle) and pindolol (-27 ± 4 mmHg, $n = 5$, $P < 0.01$) produced a transient (less than 10 min) decrease in perfusion pressure. In contrast, a slight increase in perfusion pressure ($+19 \pm 5$ mmHg, $n = 4$, $P < 0.05$) was seen after bolus injection of atenolol. Pindolol (200 μg) also caused a reduction of the vasopressin-induced coronary vasoconstriction (baseline perfusion pressure: 103 ± 21 mmHg; change: -28 ± 9 mmHg, $n = 4$).

Discussion

Our study of coronary vasodilator effects of pindolol (β_1- and β_2-blocker with partial agonistic activity) and atenolol (β_1-blocker without agonistic activity) showed that the two types of β-blocking drugs produce different effects. The concentrations of the β-blocking drugs used were chosen to produce comparable blockade of the β_1-receptor. This was shown by a similar inhibition of the isoprenaline-induced tachycardia. Despite comparable β_1-receptor block-

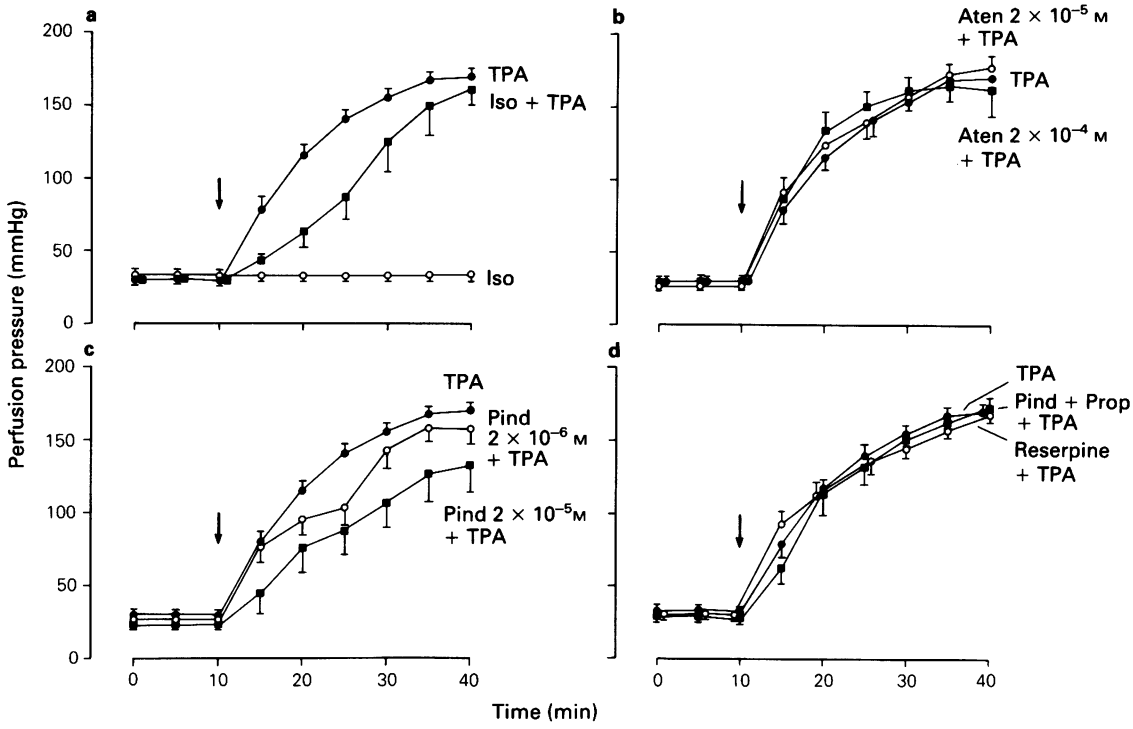

Figure 4 Effect of (a) isoprenaline (Iso), (b) atenolol (Aten), (c) pindolol (Pind) and (d) reserpine-treatment on the phorbol ester-induced increase in perfusion pressure in rat isolated perfused hearts. After a 10 min control period, drugs were added (arrows) into the perfusion fluid for 30 min. TPA = 12-0-tetradecanoyl-phorbol-13-acetate; Prop = propranolol. For other details see legend to Figure 3.

ing effects we found clear differences between the coronary haemodynamic effects of atenolol and pindolol: pindolol dose-dependently inhibited the coronary artery vasoconstriction produced by phorbol ester, while atenolol did not.

The contraction-relaxation cycle in vascular smooth muscle is regulated by changes in free myoplasmic calcium concentration. A wide variety of drugs influence calcium movements either directly or through actions of second messengers, such as cyclic GMP, cyclic AMP, inositol 1,4,5-triphosphate, diacylglycerol or calmodulin (Sasaguri *et al.*, 1987). We have previously observed that phorbol esters such as TPA, which have a structure very similar to diacylglycerol and activate protein kinase C directly (Castagna *et al.*, 1982), caused a sustained constrictor response when infused into the perfusion fluid (Ruskoaho *et al.*, 1985) similar to that described for other vascular smooth muscle preparations (Rasmussen *et al.*, 1984; Danthuluri & Deth, 1984; Forder *et al.*, 1985; Spedding, 1986). Because the rat isolated perfused heart preparation is a complex system, it remains possible that the effect of the phorbol ester on the smooth muscle cells is an indirect one mediated by neurotransmitter release.

This possibility seems unlikely as TPA-induced constriction was elicited in isolated perfused hearts obtained from reserpine-treated animals. We found that reserpine treatment which completely blocked the tyramine-induced increase in heart rate in pithed rats (Savola *et al.*, 1988) did not attenuate contraction induced by phorbol ester. In addition, prazosin (Ruskoaho, H., unpublished observation) and atenolol (this study), did not inhibit TPA-induced vasoconstriction when added directly to the perfused fluid. Thus, it seems that TPA has a direct action on the vascular smooth muscle cell rather than acting indirectly via release of endogenous noradrenaline, findings which support those of others using different vascular smooth muscle preparations (Rasmussen *et al.*, 1984; Forder *et al.*, 1985; Miller *et al.*, 1986; Itoh & Lederis, 1987).

Whether or not TPA exerts its effect via C-kinase in this preparation requires the demonstration of the

phosphorylation of specific C-kinase protein substrates. However, in our initial experiments it was possible to demonstrate that only those phorbol esters that activate protein kinase C *in vitro* increase perfusion pressure (Ruskoaho *et al.*, 1985). Thus the abilities of phorbol esters to induce coronary vasoconstriction in rat isolated perfused hearts may be related to their abilities to activate protein kinase C. In addition, our present results show that the infusion of drugs which increase or decrease the intracellular concentration of free calcium have a synergistic or antagonistic effect, respectively, on the TPA-induced coronary vasoconstriction. Bay K 8644, known to increase the influx of calcium into the cell (Reuter, 1983), caused a significant enhancement of the contractile response, whereas forskolin, which increases cyclic AMP formation and causes a fall in the intracellular free calcium concentration of vascular smooth muscle cells (Seamon & Daly, 1986), produced a marked relaxation of TPA-induced contractions. Since protein kinase C is a calcium-activated enzyme (Nishizuka, 1986), it is likely that a major site of TPA action on the vascular smooth muscle is the C-kinase.

We used the phorbol ester-induced increase in perfusion pressure in these studies as a model to study the coronary vascular effects of β-adrenoceptor blockers. The normal physiological and pharmacological responses of vascular smooth muscle to agents that induce contraction may involve a sustained activation of protein kinase C (Nishizuka, 1986; Rasmussen, 1986). To validate our model for studying a β-receptor-mediated vasodilatation, we investigated the effect of isoprenaline in combination with TPA on perfusion pressure. Infusion of isoprenaline caused a significant relaxation of the TPA-induced vascular smooth muscle contraction, showing that this preparation provides a model for studying the β-receptor-mediated vasodilatation. In our experiments, isoprenaline-induced vasodilatation was more effectively inhibited by a non-selective β-blocking drug pindolol, suggesting that the β-receptor subtype in coronary vessels is β_2. However, in doses which produced comparable inhibition of isoprenaline-induced tachycardia, atenolol also attenuated the vasodilator effect of intracoronary injection of isoprenaline. Recent *in vivo* experiments also suggest that both β_1- and β_2-receptors contribute to isoprenaline-induced vasodilatation (Vatner *et al.*, 1986).

The β-adrenoceptor-mediated coronary relaxation involves activation of adenylate cyclase catalyzing the formation of intracellular cyclic AMP, which is believed to promote relaxation in vascular smooth muscle by decreasing intracellular calcium of the cell (Sasaguri *et al.*, 1987). We found that intracoronary administration of dibuturyl-cyclic AMP, a permeable derivative of cyclic AMP, and forskolin which elevates intracellular cyclic AMP (Seamon & Daly, 1986), had essentially the same effect as isoprenaline. An increase in the myoplasmic cyclic AMP level appears thus to counteract phorbol ester-induced vasoconstriction, findings which support the concept that a bidirectional signalling system exists in vascular smooth muscle cells (Rasmussen, 1986).

The coronary haemodynamic effects resulting from β-adrenoceptor blockade are controversial. In general, β-blocking drugs are believed to be coronary vasoconstrictors (Pitt *et al.*, 1967; Yasue *et al.*, 1976; Robertson *et al.*, 1982; Kern *et al.*, 1983). Under our experimental conditions, infusion of atenolol alone increased perfusion pressure, whereas pindolol had no significant effect. Moreover, when pindolol was added to the perfusion fluid together with phorbol ester, pindolol attenuated the TPA-induced increase in perfusion pressure. The inhibitory effect of pindolol on phorbol ester-induced coronary vasoconstriction was blocked by propranolol, indicating that vasodilatation occurred as a result of β-adrenoceptor stimulation. Thus, in this model pindolol behaved as isoprenaline, having about 50% of the agonistic activity of isoprenaline (Table 5). A reduction in resistance in the femoral vascular bed in anaesthetized dogs (Clark *et al.*, 1982; Sybertz *et al.*, 1982) and in isolated mesenteric vessels of the dog (Clark *et al.*, 1982) has been demonstrated for pindolol following intra-arterial bolus injections. Intracoronary bolus injections of pindolol but not atenolol also relaxed the coronary arteries contracted by increasing perfusion rate or vasopressin.

It is difficult to assess the clinical relevance of the vasodilator effect of pindolol on angina from the results of this study. Many clinical and experimental studies of the therapeutic action of propranolol have shown a decrease in coronary flow (Berdeaux & Giudicelli, 1982; Kern *et al.*, 1983). However, intracoronary injection of propranolol in humans (Gaglione *et al.*, 1987) or propranolol and atenolol in the conscious dogs (Vatner & Hintze, 1983) did not cause coronary vasoconstriction. Thus, the reported decreases in coronary blood flow after β-adrenoceptor blockade might not be related to epicardial coronary artery vasoconstriction, but rather to secondary effects such as decrease in heart rate and contractility. In addition, recent clinical results show that exercise-induced vasoconstriction of the stenotic coronary artery can be prevented not only by intracoronary nitroglycerin (Gage *et al.*, 1986) but also by propranolol, a non-selective β-blocking drug without partial agonistic activity (Gaglione *et al.*, 1987). Whether pindolol, as a β-adrenoceptor blocking agent with coronary vasodilator properties, is clinically more beneficial than β-blockers without partial

agonistic activity, such as atenolol and propranolol, remains to be studied. This is of particular interest, because nitroglycerin further increased the effect of β-blockade suggesting that the vasodilator effect of nitroglycerin and that of β-blockade are additive (Gaglione *et al.*, 1987).

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