ELSEVIER

European Journal of Pharmacology 367 (1999) 33–39

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Reduction of myocardial injury by the EP_3 receptor agonist TEI-3356. Role of protein kinase C and of K_{ATP}-channels

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Received 29 October 1998; revised 17 December 1998; accepted 22 December 1998

Abstract

The effects of the prostanoid EP_3 receptor agonist TEI-3356 on either protein kinase C or ATP-sensitive (K_{ATP}) K^+ channels and on the infarct size caused by regional myocardial ischaemia and reperfusion in the rat were investigated. Male Wistar rats ($n = 72$) were subjected to 25 min occlusion of the left anterior descending coronary artery followed by 2 h of reperfusion. TEI-3356 (1 μg/kg/min i.v., $n = 6$) caused a significant reduction in infarct size from 60 ± 3% (control, $n = 8$) to 38 ± 3% of the area at risk. Pretreatment of rats with 5-hydroxydecanoate (5 mg/kg i.v., $n = 6$), a specific inhibitor of K_{ATP}-channels, attenuated the cardioprotective effects of TEI-3356. The reduction in infarct size afforded by TEI-3356 was also abolished by the protein kinase C inhibitors staurosporine (1 μg/kg i.v., $n = 6$) and chelerythrine (0.7 mg/kg i.v., $n = 5$). Thus, TEI-3356 reduces myocardial infarct size in the rat by a mechanism(s) which involves the activation of protein kinase C and the opening of K_{ATP}-channels.

Keywords: EP receptor; Prostaglandin, E-type; TEI-3356; Myocardial ischaemia; Reperfusion; (Rat); K^+ channel blocker; Protein kinase C

1. Introduction

There is good evidence that E-type prostaglandins exert beneficial effects in various animal models of ischaemia-reperfusion injury of the heart. For instance, E-type prostaglandines exert beneficial effects on electrocardiographical, biochemical and functional indices of ischaemia-reperfusion injury (Jugdutt et al., 1981; Schrör et al., 1988; Simpson et al., 1988). In addition, E-type prostaglandins also reduce the number of arrhythmias caused by regional myocardial ischaemia and reperfusion (Zijlstra et al., 1972; Coker and Parratt, 1983; Parratt, 1994). E-type prostaglandins reduce myocardial infarct size (Hutton et al., 1973; Jugdutt et al., 1981; Parratt, 1994; Hide et al., 1995; Hide and Thiemermann, 1996) and their effects are mediated by specific G-protein coupled receptors (EP receptors) which have been classified into four subtypes, EP_1, EP_2, EP_3 and EP_4 (Coleman et al., 1990). The cardioprotective effects of these eicosanoids may be secondary to a reduction in afterload, an increase in coronary blood flow, inhibition of platelet function and/or inhibition of the activation and extravasation of polymorphonuclear cells (Lucchesi and Mullane, 1986), all of which are secondary to the activation of EP_2 receptors, which activate G_s and cause an activation of adenylate cyclase (Coleman et al., 1990). In addition, the protection of isolated cells or organs by prostaglandins has been attributed to an ill-defined 'cytoprotective' or 'cardioprotective' effect of these agents. The mechanism(s) or the prostanoid receptor(s) mediating this effect is unknown (Schrör, 1987).

Activation of ATP-sensitive K^+ (K_{ATP}) channels reduces action potential duration, thus decreasing contractility and conserving energy during ischaemia (Nichols and Lederer, 1991; Takano and Noma, 1993). The cardioprotective effects of ischaemic preconditioning have been attributed—at least in part—to the activation and opening of K_{ATP}-channels (Parratt, 1994; Parratt and Kane, 1994). K_{ATP}-channels are found at high density in the heart (Nichols and Lederer, 1991; Takano and Noma, 1993), are opened due to activation of EP_1 or EP_3 receptors (Hide et al., 1995; Hutton et al., 1973) and mediate cardioprotective effects upon activation (Parratt and Kane, 1994). The following findings support that the K_{ATP}-pathway is in-

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0014-2999/99/$ - see front matter
PII: S0014-2999(98)00963-7

volved in the cardioprotective effects of E-type prostaglandins: (1) The cardioprotective effects of PGE_1 (non-selective agonist for all EP receptors) and sulprostone (selective agonist of EP_1 and EP_3 receptors) are abolished by inhibition of K_{ATP}-channels with glibenclamide or 5-hydroxydecanoate (Hutton et al., 1973; Hide et al., 1995). (2) Sulprostone causes cardioprotection without having any haemodynamic (EP_2 mediated) effects (Hide et al., 1995).

Previous studies have associated the activation of both K_{ATP}-channels (Parratt, 1994; Parratt and Kane, 1994) and protein kinase C (Liu et al., 1994; Speechly-Dick et al., 1994; Ytrehus et al., 1994) with the process of ischaemic preconditioning. More specifically, several investigations have suggested that K_{ATP}-channels may be a link in a signal pathway by which activation of protein kinase C triggers ischaemic preconditioning (Speechly-Dick et al., 1994; Jenkins et al., 1995). It also has been suggested that activation of EP_1 and EP_3 receptors may result in activation of protein kinase C (Coleman et al., 1990; Coleman et al., 1994). As EP_3 receptors are expressed on cardiomyocytes and are up-regulated following ischaemia of the heart (Hohlfeld, 1995; Hohlfeld et al., 1997), we have hypothesised that it is the activation of EP_3 receptors which accounts for the 'cardioprotective' and or 'cytoprotective' effects of E-type prostaglandins.

The aim of this study was to elucidate the effect(s) of TEI-3356, a highly selective agonist for the prostaglandin EP_3 receptor (Negishi et al., 1994), on the infarct size caused by regional myocardial ischaemia and reperfusion in the rat. Additionally, we have investigated the role of protein kinase C and of K_{ATP}-channels in the cardioprotective effects of this agent.

2. Methods

2.1. Myocardial ischaemia and reperfusion

Seventy-two, male Wistar rats (240–350 g, Tucks, Reyleigh, Essex, U.K.) were anaesthetised with thiopentone sodium (120 mg/kg i.p.). The trachea was cannulated and artificial respiration was maintained by a Harvard ventilator with a frequency of 70 strokes/min, a tidal volume of 8–10 ml/kg, an inspiratory oxygen concentration of 30% and a positive end-expiratory pressure of 1–2 mm Hg resulting in pCO_2 values of 36–44 mm Hg and pO_2 values over 150 mm Hg. Body temperature was maintained at 38 ± 1°C. The right carotid artery was cannulated and connected to a pressure transducer to monitor mean arterial blood pressure. The right jugular vein was cannulated for the administration of drugs. The chest was opened by a left-side thoracotomy, the pericardium was incised, a ligature was placed around the left anterior descending coronary artery using an atraumatic needle. Subdermal platinum electrodes were placed to allow the determination of a lead II electrocardiogram (ECG).

After completion of the surgical procedure, the animals were allowed to stabilise for 30 min before infusion of drugs and left anterior descending coronary artery ligation. The coronary artery was occluded at time 0, which was associated with the typical electrocardiographic (ST-segment elevation and increase in R-wave amplitude) and haemodynamic changes (fall in mean arterial blood pressure) of myocardial ischaemia. After 25 min of acute myocardial ischaemia, the ligature was re-opened to allow the reperfusion of the previously ischaemic myocardium for 2 h. Heart rate and mean arterial blood pressure were continuously recorded on a 4-channel Grass 7D polygraph recorder. The pressure rate index, a relative indicator of myocardial oxygen consumption (Baller et al., 1981), was calculated as the product of mean arterial blood pressure and heart rate, and expressed in mm Hg/min/10^3. After re-occluding the left anterior descending coronary artery Evans blue dye (1 ml of 2% w/v) was i.v. administered to separate between ischaemic (area at risk) and non-ischaemic myocardium (area not at risk). Subsequently, the heart was cut into horizontal slices to separate the area at risk from the area not at risk. Both samples were cut into small pieces and the area at risk was incubated with *p*-nitro-blue tetrazolium (NBT, 0.5 mg/ml, 20 min at

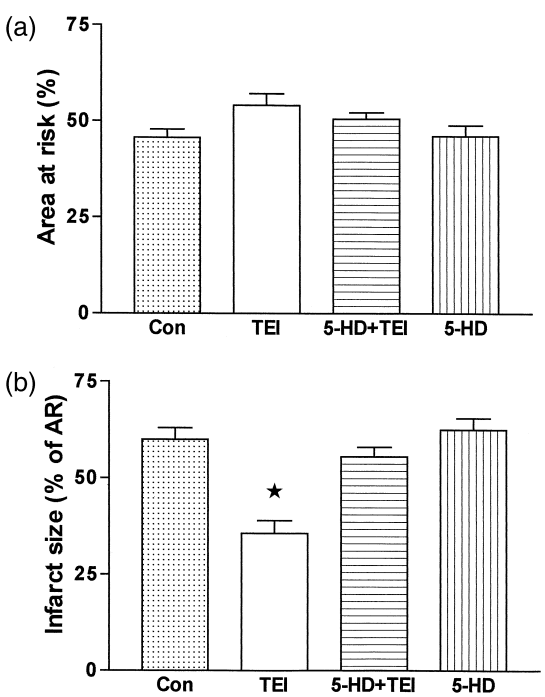

Fig. 1. Myocardial ischaemia caused by occlusion (25 min) and reperfusion (2 h) of the left anterior descending coronary artery in the anaesthetised rat. Different groups of animals were treated with vehicle (control, $n = 8$), TEI-3356 (1 μg/kg/h i.v., $n = 6$), 5-hydroxydecanoate (5-HD, 5 mg/kg i.v., $n = 6$) and 5-hydroxydecanoate plus TEI-3356 ($n = 6$). (A) Area at risk (expressed as percentage of left ventricle) in rats subjected to coronary artery occlusion. (B) Infarct size expressed as percentage of the area at risk caused by occlusion and reperfusion of the left anterior descending coronary artery. ★, $P < 0.05$ when compared to control.

37°C) to distinguish between ischaemic and infarcted tissue, while the area not at risk was incubated with saline. The area at risk and infarct size were calculated after weighing the respective tissue samples and expressed as percent of the area at risk.

2.2. Experimental groups

The following 13 experimental groups were studied to elucidate (i) the effects of the EP_3 receptor agonist TEI-3356 on the infarct size caused by regional myocardial ischaemia (25 min) and reperfusion (2 h) and (ii) the role of the activation of K_{ATP}-channels and/or protein kinase C in the cardioprotective effects of TEI-3356: (1) No occlusion of the coronary artery (sham-operation) plus infusion of vehicle (saline, $n = 3$). (2) Coronary artery occlusion (25 min) and reperfusion (2 h) plus infusion of vehicle ($n = 8$). (3) Coronary artery occlusion and reperfusion plus infusion of TEI-3356 (1 μg/kg/min, i.v. starting 10 min prior to coronary artery occlusion, $n = 6$). (4) No occlusion of the coronary artery (sham-operation) plus infusion of TEI-3356 ($n = 3$). (5) Coronary artery occlusion and reperfusion plus injection of 5-hydroxydecanoate (5 mg/kg, i.v. at 10 min prior to coronary artery occlusion, $n = 6$). (6) No occlusion of the coronary artery (sham-operation) plus injection of 5-hydroxydecanoate ($n = 3$). (7) Coronary artery occlusion and reperfusion plus administration of 5-hydroxydecanoate at 10 min prior the infusion of TEI-3356 ($n = 6$). (8) Coronary artery occlusion and reperfusion plus injection of staurosporine (1 μg/kg i.v. 10 min prior to coronary artery occlusion, $n = 6$). (9) No occlusion of the coronary artery (sham-operation) and injection of staurosporine ($n = 3$). (10) Coronary artery occlusion and reperfusion plus administration of staurosporine 10 min prior the infusion of TEI-3356 ($n = 6$). (11) Coronary artery occlusion and reperfusion plus injection of chelerythrine (0.7 mg/kg i.v. 10 min prior to coronary artery occlusion, $n = 6$). (12) No occlusion of the coronary artery (sham-operation) and injection of chelerythrine ($n = 3$). (13) Coronary artery occlusion and reperfusion plus administration of chelerythrine at 10 min prior the infusion of TEI-3356 ($n = 5$).

The *n*-numbers in the above experimental groups refer to animals, which survived until the end of the experiment. The number of animals which died in the individual groups of animals studied were the following: (1) $n = 1$; (3) $n = 1$; (5) $n = 1$; (7) $n = 1$; (8) $n = 1$; (11) $n = 1$ and (13) $n = 2$.

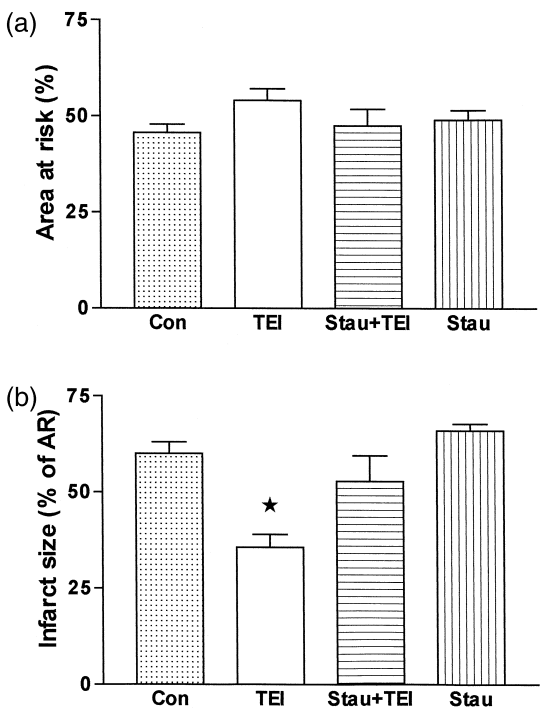

Fig. 2. Myocardial ischaemia caused by occlusion (25 min) and reperfusion (2 h) of the left anterior descending coronary artery in the anaesthetised rat. Different groups of animals were treated with vehicle (control, $n = 8$), TEI-3356 (1 μg/kg/h i.v., $n = 6$), staurosporine (Stau, 1 μg/kg i.v., $n = 6$) and staurosporine plus TEI-3356 ($n = 6$). (A) Area at risk (expressed as percentage of left ventricle) in rats subjected to coronary artery occlusion. (B) Infarct size expressed as percentage of the area at risk caused by occlusion and reperfusion of the left anterior descending coronary artery. ★, $P < 0.05$ when compared to control.

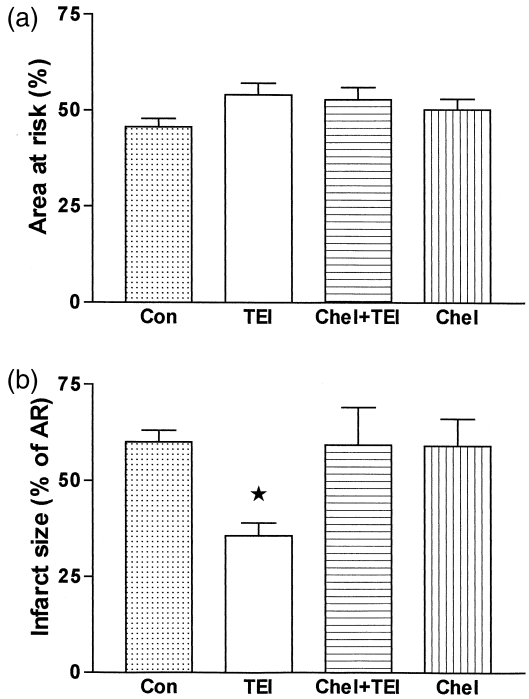

Fig. 3. Myocardial ischaemia caused by occlusion (25 min) and reperfusion (2 h) of the left anterior descending coronary artery in the anaesthetised rat. Different groups of animals were treated with vehicle (control, $n = 8$), TEI-3356 (1 μg/kg/h i.v., $n = 6$), chelerythrine (Chel, 0.7 mg/kg i.v., $n = 6$) and chelerythrine plus TEI-3356 ($n = 5$). (A) Area at risk (expressed as percentage of left ventricle) in rats subjected to coronary artery occlusion. (B) Infarct size expressed as percentage of the area at risk caused by occlusion and reperfusion of the left anterior descending coronary artery. ★, $P < 0.05$ when compared to control.

2.3. Drugs and materials

Chelerythrine and staurosporine were solved in dimethyl sulfoxide (DMSO) (final concentration in vivo less than 0.2% for DMSO), TEI-3356 was solved in 5% ethanol and 0.5% Twin 80 and saline (final concentration in vivo less than 0.1% for ethanol and 0.01% for Twin 80). We have recently shown that PGE_1 and sulprostone significantly reduced myocardial infarct size in vivo without causing haemodynamic effects when they were administered as continuous infusions of 1 μg/kg/min (Hide and Thiemermann, 1996). As TEI-3356 has the same potency as an EP_3

Table 1
Mean arterial pressure (MAP, mm Hg), heart rate (HR, beats/min) and pressure rate index (PRI, mm Hg/min/10^3) in rats subjected to 25 min of coronary artery occlusion and 2 h reperfusion

Group		Treatment (min)		Occlusion (min)			Reperfusion (min)	
		−20	−10	0	15	25	60	120
Control ($n=8$)	MAP	123 ± 9	122 ± 7	122 ± 8	122 ± 8	116 ± 6	93 ± 6	87 ± 4
	HR	458 ± 11	458 ± 11	454 ± 11	454 ± 11	450 ± 11	454 ± 12	445 ± 14
	PRI	56 ± 5	56 ± 4	56 ± 4	56 ± 5	52 ± 4	42 ± 2	39 ± 3
Sham control ($n=3$)	MAP	127 ± 29	120 ± 23	117 ± 20	115 ± 17	103 ± 13	98 ± 5	102 ± 9
	HR	420 ± 0	410 ± 10	400 ± 10	400 ± 10	380 ± 20	340 ± 20[a]	370 ± 26
	PRI	53 ± 12	49 ± 10	46 ± 7	46 ± 6	39 ± 4	33 ± 1	37 ± 1
TEI-3356 ($n=6$)	MAP	125 ± 7	117 ± 7	113 ± 7	97 ± 6	95 ± 8	103 ± 6	108 ± 8
	HR	415 ± 5	410 ± 10	400 ± 10	405 ± 15	400 ± 13	395 ± 16	410 ± 23
	PRI	52 ± 3	48 ± 4	46 ± 4	40 ± 4	38 ± 4	41 ± 2	44 ± 5
Sham TEI-3356 ($n=3$)	MAP	120 ± 10	107 ± 7	107 ± 7	100 ± 6	97 ± 3	93 ± 3	85 ± 5
	HR	450 ± 17	420 ± 17	420 ± 17	410 ± 26	400 ± 20	390 ± 17	410 ± 36
	PRI	54 ± 7	45 ± 5	45 ± 5	41 ± 4	39 ± 3	37 ± 3	35 ± 1
5-HD ($n=6$)	MAP	127 ± 10	124 ± 10	119 ± 9	112 ± 9	102 ± 10	98 ± 8	96 ± 5
	HR	425 ± 20	425 ± 20	425 ± 20	430 ± 23	430 ± 20	415 ± 20	435 ± 24
	PRI	54 ± 5	53 ± 6	51 ± 5	48 ± 3	43 ± 4	41 ± 4	41 ± 2
Sham 5-HD ($n=3$)	MAP	120 ± 12	113 ± 9	113 ± 9	99 ± 6	93 ± 3	95 ± 3	97 ± 4
	HR	450 ± 17	450 ± 17	460 ± 10	440 ± 20	430 ± 10	410 ± 10	410 ± 10
	PRI	54 ± 7	51 ± 6	52 ± 5	44 ± 5	40 ± 2	39 ± 1	40 ± 2
5-HD + TEI-3356 ($n=6$)	MAP	129 ± 4	129 ± 5	126 ± 5	107 ± 6	102 ± 4	91 ± 3	87 ± 4
	HR	425 ± 5	425 ± 5	425 ± 5	410 ± 6	410 ± 6	390 ± 8	395 ± 14
	PRI	55 ± 2	55 ± 2	53 ± 2	44 ± 3	42 ± 2	36 ± 2	34 ± 2
Staurosporine ($n=6$)	MAP	103 ± 6	99 ± 6	97 ± 5	94 ± 4	88 ± 2	89 ± 5	91 ± 4
	HR	425 ± 12	420 ± 13	425 ± 12	430 ± 17	430 ± 17	420 ± 20	470 ± 10
	PRI	44 ± 2	42 ± 3	41 ± 3	40 ± 2	38 ± 2	38 ± 3	42 ± 1
Sham staurosporine ($n=3$)	MAP	103 ± 7	101 ± 10	98 ± 8	91 ± 5	93 ± 9	87 ± 3	90 ± 0
	HR	410 ± 36	410 ± 36	410 ± 36	400 ± 40	420 ± 35	410 ± 36	410 ± 36
	PRI	43 ± 6	42 ± 8	41 ± 7	37 ± 6	39 ± 4	35 ± 2	37 ± 3
Staurosporine + TEI-3356 ($n=6$)	MAP	108 ± 6	100 ± 7	97 ± 6	93 ± 6	93 ± 7	83 ± 4	90 ± 2
	HR	430 ± 10	415 ± 16	415 ± 12	415 ± 14	405 ± 10	405 ± 17	415 ± 33
	PRI	46 ± 3	42 ± 4	40 ± 3	39 ± 3	38 ± 3	34 ± 1	37 ± 3
Chelerythrine ($n=6$)	MAP	101 ± 7	102 ± 8	102 ± 8	89 ± 9	83 ± 7	88 ± 5	83 ± 4
	HR	435 ± 17	430 ± 13	430 ± 13	435 ± 10	430 ± 13	440 ± 6	435 ± 7
	PRI	43 ± 2	44 ± 3	44 ± 3	38 ± 3	36 ± 3	39 ± 2	36 ± 2
Sham chelerythrine ($n=3$)	MAP	138 ± 15	133 ± 12	133 ± 12	127 ± 13	121 ± 12	108 ± 15	101 ± 12
	HR	440 ± 26	430 ± 36	430 ± 36	420 ± 35	420 ± 35	420 ± 17	420 ± 30
	PRI	62 ± 10	58 ± 10	58 ± 10	54 ± 10	52 ± 9	46 ± 8	43 ± 8
Chelerythrine + TEI-3356 ($n=6$)	MAP	118 ± 6	116 ± 6	110 ± 7	101 ± 8	101 ± 11	87 ± 9	72 ± 10
	HR	420 ± 9	420 ± 9	420 ± 9	420 ± 0	426 ± 6	444 ± 15	462 ± 26
	PRI	50 ± 2	49 ± 2	46 ± 2	43 ± 4	43 ± 5	39 ± 4	32 ± 3

Data are mean ± S.E.M.
[a] $P < 0.05$ when compared to control. 5-HD: 5-hydroxydecanoate.
TEI-3356 was administered as a continuous infusion (1 μg/kg/min i.v.) starting 10 min prior to coronary artery occlusion and continued until the end of reperfusion.
5-Hydroxydecanoate (5-HD) was administered as an i.v. bolus (5 mg/kg i.v.) 10 min before onset of the TEI-3356 infusion.
The protein kinase C inhibitors staurosporine (1 μg/kg) or chelerythrine (0.7 mg/kg) were administered as an i.v. bolus injection 10 min before starting the infusion of TEI-3356.
In addition, sham-operation (no coronary artery-occlusion) and administration of saline, TEI-3356, 5-hydroxydecanoate, staurosporine or chelerythrine was carried out.
★, $P < 0.05$ when compared to vehicle control.

receptor agonist as PGE_2 or sulprostone (Negishi et al., 1994), we used a dose of 1 μg/kg/min of TEI-3356 in vivo. Unfortunately, the volume distribution and half live of TEI-3356 in vivo are not known. Unless otherwise stated, all compounds were obtained from Sigma (Poole, Dorset, U.K). Thiopentone sodium (Intraval®) was obtained from May and Baker (Dagenham, U.K). Chelerythrine and staurosporine were from Calbiochem (Nottingham, U.K). We thank Dr. Atsuo Hazato (Teijin, Tokyo, Japan) for the generous supply of TEI-3356.

2.4. Statistical analysis

All values in the text, figures and table are expressed as the mean ± S.E.M. of n observations. Statistical analysis was performed (on absolute values) by one-way analysis of variance (ANOVA) followed, if appropriate, by a Bonferroni's test for multiple comparisons. A P value of less than 0.05 was considered statistically significant.

3. Results

3.1. The cardioprotective effects of TEI-3356 are antagonised by 5-hydroxydecanoate

The mean values for the areas at risk were similar in all animal groups studied (Fig. 1a, $P > 0.05$). In rats which had received an infusion of the vehicle for the EP_3 receptor agonist, occlusion of the left anterior descending coronary artery (for 25 min) followed by reperfusion (for 2 h) resulted in an infarct size of 60 ± 3% of the area at risk (control, $n = 8$). When compared to vehicle, infusion of TEI-3356 ($n = 6$) caused a significant reduction in infarct size of approximately 38% (Fig. 1b).

Pretreatment of rats with the K_{ATP}-channel blocker 5-hydroxydecanoate abolished the reduction in infarct size afforded by the subsequent infusion of TEI-3356 (Fig. 1b, $n = 6$, $P > 0.05$ compared to control). However, pretreatment of rats with 5-hydroxydecanoate prior to commencing the infusion of vehicle for the EP_3 receptor agonist did not result in a significant reduction in myocardial infarct size (Fig. 1b).

3.2. The cardioprotective effects of TEI-3356 are abolished by staurosporine and chelerythrine

The mean values for the areas at risk were similar in all animal groups studied (Fig. 2a and b, $P > 0.05$). Pretreatment of rats with the protein kinase C inhibitor staurosporine also abolished the reduction in infarct size afforded by TEI-3356 (Fig. 2b), while staurosporine did not affect infarct size when given to rats treated with vehicle for TEI-3356 (Fig. 2b). Pretreatment of rats with the protein kinase C inhibitor chelerythrine also abolished the reduction in infarct size afforded by TEI-3356 (Fig. 3b), while chelerythrine did not affect infarct size when given to rats treated with vehicle for TEI-3356 (Fig. 3b).

Sham-operation did not result in a significant degree of infarction in any of the groups studied (less than 3% of the area at risk, data not shown). In addition, DMSO, ethanol and Twin 80 (in the concentrations used in this study) did not affect infarct size in rats subjected to regional myocardial ischaemia and reperfusion (data not shown).

3.3. Haemodynamic effects of TEI-3356 in rats subjected to myocardial ischaemia and reperfusion

Values for mean arterial blood pressure, heart rate and pressure rate index measured during the course of the experiments are given in Table 1. Baseline haemodynamic data (e.g., mean arterial blood pressure, heart rate and pressure rate) were similar ($P > 0.05$) in all groups studied. In sham-operated rats (no coronary artery occlusion), infusion of vehicle (saline), 5-hydroxydecanoate, TEI-3356, staurosporine or chelerythrine did not cause any significant effects on mean arterial blood pressure or pressure rate index. In one group studied (sham vehicle control), the mean value for heart rate was, however, at 60 min into the reperfusion period lower than in the control group and recovered at the end of the experiment. In rats subjected to coronary artery occlusion and reperfusion which received an infusion of saline (control, $n = 8$), mean values for mean arterial blood pressure and pressure rate index fell throughout the experimental period, but there was no alteration in heart rate (Table 1). TEI-3356 did not affect mean arterial blood pressure, heart rate or pressure rate index in rats subjected to coronary artery occlusion and reperfusion. Pretreatment of rats subjected to coronary artery occlusion and reperfusion with 5-hydroxydecanoate, staurosporine or chelerythrine did also not result in any significant haemodynamic changes (Table 1).

4. Discussion

4.1. TEI-3356, a specific agonist of the prostanoid EP_3 receptor, reduces myocardial infarct size without causing haemodynamic effects

This study demonstrates that the specific prostanoid EP_3 receptor agonist TEI-3356 (Negishi et al., 1994) reduces the myocardial infarct size caused by coronary artery occlusion and reperfusion in the anaesthetised rat without causing haemodynamic effects.

A reduction in blood pressure and, hence, myocardial oxygen consumption (EP_2 mediated) does not account for the cardioprotective effects of TEI-3356, as this EP_3 receptor agonist did not cause any significant reduction in blood pressure or pressure-rate index. There is a strong positive correlation between myocardial oxygen consumption and pressure-rate index (Baller et al., 1981) and, hence, the

observed cardioprotective effect of TEI-3356 is not likely to be secondary to a reduction in myocardial oxygen demand.

The observation that TEI-3356 did not reduce blood pressure in the rat also confirms that (at the dose used in this study) TEI-3356 did not activate EP_2 receptors in vivo, activation which results in stimulation of G_s, an increase in cAMP and vasodilatation (Coleman et al., 1990).

We therefore propose that the observed cardioprotective effects are due to the activation of EP_3 receptors, which are up-regulated during myocardial ischaemia (Hohlfeld, 1995; Hohlfeld et al., 1997). In order to gain a better insight into the mechanism(s) by which the EP_3 receptor agonist TEI-3356 reduces myocardial infarct size in the rat, we have investigated the signal transduction events underlying the observed cardioprotective effects.

4.2. Role of ATP-sensitive K^+ channels in the cardioprotective effects of TEI-3356

The reduction in infarct size caused by either PGE_1 or by the EP_1/EP_3 receptor agonist sulprostone is (at least in part) due to the activation and opening of K_{ATP}-channels (Hide et al., 1995; Hide and Thiemermann, 1996). Although glibenclamide is not a specific inhibitor of cardiac K_{ATP}-channels, both glibenclamide and 5-hydroxydecanoate have been used to document that the cardioprotective effects of ischaemic preconditioning involve the activation of K_{ATP}-channels (Hide and Thiemermann, 1996). It has been proposed that the opening of mitochondrial K_{ATP}-channels mediates the cardioprotective effects of ischaemic preconditioning (Paucek et al., 1995; Garlid et al., 1996). In comparison with glibenclamide, a non-specific inhibitor of K_{ATP}-channels (e.g., inhibits sarcolemmal and mitochondrial K_{ATP}-channels), 5-hydroxydecanoate selectively blocks mitochondrial K_{ATP}-channels (Garlid et al., 1997).

Interestingly 5-hydroxydecanoate attenuates the reduction in infarct size afforded by ischaemic preconditioning (Hide and Thiemermann, 1996). In this study we report that the cardioprotective effect of TEI-3356 is also abolished by pretreatment of rats with 5-hydroxydecanoate. This finding suggest that TEI-3356 (and presumably the activation of EP_3 receptors) leads to opening of mitochondrial K_{ATP}-channels which, in turn, results in cardioprotection. The mechanism by which opening of K_{ATP}-channels protects the myocardium against ischaemic injury is not clear.

4.3. Role of protein kinase C in the cardioprotective effects of TEI-3356

Numerous studies support the hypothesis that K_{ATP}-channels may be linked to a signal pathway by which activation of protein kinase C triggers ischaemic preconditioning (Speechly-Dick et al., 1994; Jenkins et al., 1995). Recently it has been shown that protein kinase C can directly activate single cardiac K_{ATP}-channels at physiological levels of ATP (Light et al., 1996). It appears likely that K_{ATP}-channels can be regulated by several intracellular signalling pathways, which act via protein kinase C to alter the channel activity. The signal transduction events involved in the cardioprotective effects of TEI-3356 are reminiscent of those that mediate the potent anti-ischaemic effects of 'ischaemic preconditioning' (Downey and Cohen, 1995). Having demonstrated that the cardioprotective effects of TEI-3356 are abolished by 5-hydroxydecanoate, we have investigated the potential role of protein kinase C in the observed cardioprotective effects of this EP_3 receptor agonist. We demonstrate here that the reduction in infarct size afforded by TEI-3356 in the rat is abolished by two inhibitors of protein kinase C, namely staurosporine and chelerythrine, which inhibit the activation of protein kinase C in vivo and ex vivo (Speechly-Dick et al., 1994; Kaye et al., 1995; Kozak et al., 1997) and also abolish the cardioprotective effects of 'ischaemic preconditioning' in rodents in vivo (Speechly-Dick et al., 1994; Yoshida et al., 1997). These findings suggest that the signal transduction events leading to a reduction in infarct size caused by TEI-3356 involve the activation of protein kinase C and finally the opening of K_{ATP}-channels.

4.4. Conclusion

In conclusion, this study demonstrates for the first time that the prostanoid EP_3 receptor agonist TEI-3356 reduces infarct size in rats subjected to regional myocardial ischaemia and reperfusion. These results imply that selective agonists of the prostanoid EP_3 receptor may be useful to protect the heart against ischaemia-reperfusion injury without causing any significant haemodynamic effects.

The mechanism(s) of the cardioprotective effects of this agent is not entirely clear, but may involve the activation of protein kinase C and the opening of mitochondrial K_{ATP}-channels via EP_3 receptor.

Acknowledgements

KZ is supported by the Deutsche Gesellschaft für Kardiologie-Herz-und Kreislaufforschung. AO is supported by the Deutsche Forschungsgemeinschaft (OL 109/1-2). CT is a Senior Fellow of the British Heart Foundation (BHF FS 96/018).

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