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FP-receptor mediated trophic effects of prostanoids in rat ventricular cardiomyocytes

[1]K. Pönicke, [1]C. Giessler, [1]M. Grapow, [1]I. Heinroth-Hoffmann, [1]K. Becker, [2]B. Osten & *,[1]O.-E. Brodde

[1]Institute of Pharmacology and Toxicology, Martin-Luther-University of Halle-Wittenberg, Magdeburger Str. 4, D-06097 Halle (Saale), Germany and [2]Department of Nephrology, Ernst Grube Str. 40, D-06120 Halle (Saale), Germany

1 The aim of this study was to characterize the receptor subtype involved in cardiac effects of prostanoids. For this purpose we determined in neonatal and adult rat cardiomyocytes effects of prostanoids on inositol phosphate (InsP)-formation (assessed as accumulation of total [^{3}H]-InsP's in myo-[^{3}H]-inositol pre-labelled cells) and on rate of protein synthesis (assessed as [^{3}H]-phenylalanine incorporation), and on contractile force in left ventricular strips of the rat heart. For comparison, effects of prostanoids on InsP-formation and contractile force were determined in rat thoracic aorta, a classical TP-receptor containing tissue.

2 Prostanoid increased InsP-formation and rate of protein synthesis in neonatal as well as adult rat cardiomyocytes; the order of potency was in neonatal ($PGF_{2\alpha} > PGD_2 \geqslant PGE_2 \geqslant$ U 46619 $> PGE_1$) and adult ($PGF_{2\alpha} > PGD_2 \geqslant PGE_2 >$ U 46619) rat cardiomyocytes well comparable. Moreover, in electrically driven left ventricular strips $PGF_{2\alpha}$ caused positive inotropic effects (pD_2 7.5) whereas U 46619 (up to 1 μM) was uneffective.

3 In contrast, in rat thoracic aorta U 46619 was about 100 times more potent than $PGF_{2\alpha}$ in increasing InsP-formation and contractile force.

4 The TP-receptor antagonist SQ 29548 only weakly antagonized prostanoid-induced increases in rate of protein synthesis (pK_B about 6) in rat cardiomyocytes but was very potent (pK_B about 8–9) in antagonizing prostanoid-induced increases in InsP-formation and contractile force in rat aorta.

5 We conclude that, in cardiomyocytes of neonatal and adult rats, the prostanoid-receptor mediating increases in InsP-formation and rate of protein synthesis is a FP-receptor. Moreover, stimulation of these cardiac FP-receptors can mediate increases in contractile force.
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Keywords: Prostanoid-receptors; neonatal rat cardiomyocytes; adult rat cardiomyocytes; protein synthesis; inositol phosphate; hypertrophy; contractile force

Abbreviations: DAG, diacylglycerol; ET-1, endothelin-1; InsP, inositol phosphate; PG, prostaglandin; PKC, protein kinase C; PLC, phospholipase C; TXA_2, thromboxane A_2

Introduction

It is now generally accepted that stimulation of receptors that couple through $G_{q/11}$-protein to phospholipase C (PLC) leads to formation of the second messengers inositol trisphosphate ($InsP_3$) and diacylglycerol (DAG). DAG can activate certain isoforms of protein kinase C (PKC) and growing evidence has accumulated that this pathway is involved in induction of cell growth in various cell systems including cardiac myocytes (for recent review see Sugden & Clerk, 1998; Dorn & Brown, 1999). Thus, several groups have convincingly shown that, in neonatal rat ventricular cardiomyocytes, stimulation of α_1-adrenoceptors (Simpson, 1983; Lee *et al*., 1988; Knowlton *et al*., 1993) and ET_A-receptors (Shubeita *et al*., 1990; Suzuki *et al*., 1990; Sugden *et al*., 1993; Ito *et al*., 1993, for review see Sugden & Bogoyevitch, 1995) causes increases in rate of protein synthesis. We have recently shown, that in these cells also the TXA_2-mimetic U 46619 increased InsP-formation and rate of protein synthesis (Pönicke *et al*., 1999); the effects of U 46619 appeared, however, not to be mediated by a TP-receptor, since the TP-receptor antagonist SQ 29548 (Ogletree *et al*., 1985) was only a weak antagonist of these effects with a potency (pK_B-value around 6) 100 times less than could be expected for a TP-receptor mediated effect (7.5–9.1, Coleman *et al*., 1994).

The aim of the present study was, therefore, to find out which prostanoid-receptor subtype might be involved in the growth-promoting effects of U 46619 in the rat cardiomyocytes. For this purpose we firstly studied the effects of a series of prostanoids on InsP-formation and rate of protein synthesis (assessed by [^{3}H]-phenylalanine incorporation) in neonatal rat cardiomyocytes. We secondly repeated these studies in ventricular cardiomyocytes isolated from adult rats in order to find out whether or not prostanoid receptors in neonatal cardiomyocytes resemble those in adult cardiomyocytes. And thirdly for reason of comparison we studied the effects of several prostanoids on InsP-formation and vasoconstriction in rat thoracic aorta, a tissue widely used to study TP-receptor-mediated effects (Jones *et al*., 1989; Tymkewycz *et al*., 1991; Wagner *et al*., 1997).

Methods

Preparation of neonatal rat cardiomyocyte culture

Cardiomyocytes of neonatal rats were isolated as described recently (Pönicke *et al*., 1997). In order to prevent proliferation

*Author for correspondence;
E-mail: otto-erich.brodde@medizin.uni-halle.de

of non-myocytes (mainly fibroblasts) and to obtain cardiomyocytes that were nearly free of contamination, cells were prepared and cultured in presence of 10 μM cytosine-β-D-arabinofuranoside.

Preparation of cardiomyocyte culture of adult rats

Cardiomyocytes of adult rats were isolated as described by Piper & Volz (1990) in a slightly modified version. Briefly, 12 weeks old male Wistar rats were anaesthetized with pentobarbitone sodium (35 mg kg^{-1}, i.p.) and 500 U heparin sulphate were injected intraperitoneally. The heart was rapidly excised under artificial ventilation. The calcium-tolerant myocytes were isolated by cardiac retrograde aortic perfusion (Langendorff method) as described by Viko *et al.* (1995). Freshly isolated left ventricular cells were gently diluted in steril culture medium 199, pH 7.4, supplemented with 10% new-born calf serum. For studies of InsP-formation the resultant suspension of ventricular myocytes was transferred into 75 cm^2 cell culture flasks (3.6×10^4 cells cm^{-2}) and immediately used. For studies of [^{3}H]-phenylalanine incorporation the ventricular myocytes suspension was seeded into 12-well-plates (16,000 cells per well) which had been coated with 4% foetal calf serum in medium 199 for 24 h at 37°C (in a humidified incubator at 5% CO_2/95% air) and incubated for 16 h at 37°C. Thereafter, the cultures were rinsed with serum-free Hank's balanced salt solution to remove damaged, rounded and nonattached myocytes and the rod-shaped cells were cultured for the following experiments in serum-free medium M199 supplemented with 2 mM L-carnitine, 5 mM taurine, 5 mM creatine and antibiotics (100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin). To prevent growth of nonmyocytes, the culture medium was supplemented with 10 μM cytosine-β-D-arabinofuranoside.

Incorporation of [^{3}H]-phenylalanine

Protein synthesis by cardiomyocytes was assessed by incorporation of [^{3}H]-phenylalanine into cells as recently described (Pönicke *et al.*, 1997). Briefly, after addition of [^{3}H]-phenylalanine (0.5 μCi ml^{-1}) at 37°C and various concentrations of prostanoids in the presence or absence of antagonists the cultures were incubated for 20 h at 37°C in 5% CO_2/95% air. At the end of the experiments cells were washed with ice-cold 0.9% NaCl-solution and incubated for 24 h at 4°C with 10% trichloroacetic acid. Thereafter precipitates were washed again with 10% trichloroacetic acid and twice with 0.9% NaCl solution. The remaining precipitate on the culture dishes was solubilized in 1 N NaOH supplemented with 0.1% sodium dodecyl sulphate by room temperature for 24 h, and radioactivity was determined in aliquots by the use of a liquid scintillation counter (Beckman LS 6000).

Inositol phosphate formation in neonatal rat cardiomyocytes

InsP-formation in rat cardiomyocytes was determined as recently described (Pönicke *et al.*, 1997). Briefly, after the 24 h incubation (see above) cells were washed with culture medium M199 supplemented with 10% new-born calf serum and 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin and incubated for 24 h with myo-[^{3}H]-inositol (2.9 μCi ml^{-1}) at 37°C. Thereafter adherent cells were peeled off by trypsin-EDTA treatment and non-incorporated myo-[^{3}H]-inositol was washed out by centrifugation and resuspension in Hanks' buffered saline solution supplemented with 10 mM LiCl and 1% bovine serum albumin. Aliquots (970 μl) of the cardiomyocyte suspension (2.3×10^5 cells ml^{-1}) were then incubated with the indicated prostanoids in the presence or absence of antagonists for 60 min at 37°C in a final volume of 1 ml. The incubation was stopped by addition of 1 ml ice-cold methanol and 2 ml chloroform. Total inositol phosphates were separated and determined as recently described (Pönicke *et al.*, 1997). Each data point was determined in quadruplicate in each experiment.

Inositol phosphate formation in adult rat cardiomyocytes

The ventricular myocytes suspension (see above) was incubated for 24 h with myo-[^{3}H]-inositol (2.9 μCi ml^{-1}) at 37°C. Thereafter, non-incorporated myo-[^{3}H]-inositol was washed out by centrifugation and resuspension in Hanks buffered saline solution supplemented with 10 mM LiCl and 1% bovine serum albumin. Aliquotes (970 μl) of the cardiomyocyte suspension (5×10^4 cells ml^{-1}) were incubated with the indicated prostanoids in a final volume of 1 ml for 45 min at 37°C. Total inositol phosphates were separated and determined as described above.

Inositol phosphate formation in rat ventricular slices

InsP-formation in ventricular slices of rat heart was determined as recently described (Pönicke *et al.*, 1998).

Inositol phosphate formation in rat aortic rings

Male Wistar rats (12 weeks old) were killed by cervical dislocation; the thoracic aorta was removed rapidly and placed into oxygenated Krebs-Henseleit buffer of the following composition (mM): NaCl 108, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 24.9, $CaCl_2$ 1.3, D-glucose 11, EDTA 0.001. Fat surrounding the aorta was carefully removed without stretching the tissue. The aorta was cleaned of adhering fat and connective tissue and cut into rings of 1 mm wide with a tissue shopper. The rings were maintained at 37°C and continuously bubbled with carbogen for 45 min. In this time the buffer was changed twice. Thereafter the aortic rings were preincubated with myo-[^{3}H]-inositol (6 μCi ml^{-1}) for 1 h. After washout of the radioactivity, three rings of each aorta were incubated in a total volume of 330 μl in the presence or absence of prostanoids for 45 min at 37°C. The incubation was stopped by addition of 330 μl ice-cold methanol and 660 μl chloroform. The mixture was vigorously vortexed twice, and thereafter the phases were separated by centrifugation at $820 \times g$ for 10 min at 4°C. Aliquots (400 μl) of the upper phase were placed on Dowex AG 1-X8 columns (200 mg per column). Free inositol was eluted twice each with 5 ml H_2O and 5 ml of 60 mM ammonium formate. Total inositol phosphates were eluted by addition of 2×1 ml 1 M ammonium formate dissolved in 100 mM formic acid. Each data point was determined in quadruplicate in each experiment.

Preparation of rat ventricular strips

The preparation of ventricular strips of the rat heart were performed as described earlier (Kotchi Kotchi *et al.*, 1998). Briefly, male Wistar rats (12 weeks old) were killed by cervical dislocation; the hearts were rapidly removed, placed into oxygenated modified Tyrode solution containing (mM): NaCl 136.9, KCl 5.4, NaH_2PO_4 0.42, MgCl 1.05, $NaHCO_3$ 25.0, $CaCl_2$ 2.5, D-glucose 9.7 equilibrated with carbogen, and

trabecular strips (1–2 mm wide, 1–1.5 mm thick, 6–8 mm long) were prepared from the left ventricle. The ventricular strips were mounted in a 10 ml organ bath containing Tyrode solution at 37°C. The strips were electrically stimulated by square wave pulse (5 ms) of about 50% above threshold at a frequency of 1 Hz (Stimulator II, Hugo Sachs Elektronik KG, March-Hugstetten, Germany). The developed tension of the preparation (maintained under a resting tension of 10 mN) was recorded *via* a strain gauge on a Hellige recorder (Hellige GmbH Freiburg, Germany). After an equilibration time of at least 1 h, the cumulative concentration-response curves for prostanoids were determined.

Preparation of rat aortic strips

Male Wistar rats (12 weeks old) were killed by cervical dislocation. The thoracic aorta was rapidly removed and placed into oxygenated modified Krebs-Henseleit solution containing (mM): NaCl 119, KCl 4.75, KH_2PO_4 1.19, $MgSO_4$ 1.19, $NaHCO_3$ 25, $CaCl_2$ 2.25, D-glucose 10, EDTA 0.0228, ascorbic acid 0.117. The aorta was cleaned of adhering fat and connective tissue, and cut into helical strips 2 mm in width and 10 mm in length. The strips were placed in 10 ml chambers containing Krebs-Henseleit solution with constant oxygenation (carbogen) at 37°C. The contractile force was measured isometrically using force transducers connected to amplifiers and recorders (Föhr Medical Instruments GmbH, Germany). The resting tension in the aorta was adjusted to 9.81 mN. The strips were allowed to equilibrate for 60 min (bath fluid was replaced at 20 min intervals). Following equilibration, the aortic strips were contracted by treatment with 50 mM KCl and 1 μM phenylephrine to confirm the viability of the smooth muscle. When tension had reached a plateau, 10 μM carbachol was added to verify the functional state of the endothelium. The bath was then washed repeatedly with Krebs-Henseleit solution until the preparations reached their initial tension. After this equilibration period, cumulative concentration-response curves for the prostanoids were determined. For SQ 29548 experiments, aortic strips were incubated for 30 min with SQ 29548 in the indicated concentrations; thereafter cumulative concentration-response curves for prostanoids were determined in presence of SQ 29548.

Drugs

L-[2,3,4,5,6a[^{3}H]-phenylalanine (spec. Activity: 5.03 TBq mmol^{-1}) and myo-[^{3}H]-inositol (spec. activity: 4.25 TBq mmol^{-1}) was purchased from Amersham Buchler (Braunschweig, Germany). $PGF_{2\alpha}$, PGD_2, PGE_1, PGE_2 were purchased from Saxon Biochemicals (Hannover, Germany), carbocyclin from Calbiochem (Bad Soden, Germany) and L-phenylalanine, cytosine-β-D-arabinofuranoside, sodium dodecyl sulphate, trypsin (crude), L-carnitine, taurine, creatine from Sigma-Aldrich (Deisenhofen, Germany). Hanks' balanced salt solutions, culture medium M199 and penicillin-streptomycin were obtained from Life Technologies (Eggenstein, Germany). U 46619 (9,11-dideoxy-11α,9α-epoxy-methanoprostaglandin $F_{2\alpha}$) and SQ 29548 [{1S-(1α,2α(Z),3α,4α)}-7-{3-[[2-[(phenylamino)carbonyl]hydrazino]methyl]-7-oxabicyclo[2.2.1]hept-2-yl}-5-heptanoic acid] were purchased from Reatec GmbH (Weiterstadt, Germany). All other chemicals were of the highest purity grade commercially available.

Statistical analysis

Data given are means ± s.e.mean of n experiments. Agonist-concentration-effect curves were fitted to sigmoid function with fixed slopes at 1.0, and EC_{50}-values were calculated by non-linear regression analysis using the GraphPad Prism 2.01 program (GraphPad Software, San Diego, U.S.A.). In order to find out whether or not, in rat aorta, concentration-effect curves for $PGF_{2\alpha}$ effects on InsP-formation and contraction fitted better to a two-site model or to a one-site model the F-test was used (GraphPad Prism 2.01 program, GraphPad Software, San Diego, U.S.A.).

The apparent SQ 29548 affinity was either calculated using the formula

$$K_B = A/(CR - 1)$$

(concentration-experiments on helically cut strips of aorta) with A is the concentration of SQ 29548, and CR is the ratio of EC_{50}-values of agonist measured in the presence and absence of SQ 29548 or using the Cheng & Prusoff-equation (Cheng & Prusoff, 1973)

$$K_i = IC_{50}/([S]/EC_{50}) + 1$$

(InsP-formation experiments on aortic rings) with IC_{50} concentration of SQ 29548 to inhibit agonist-induced InsP-

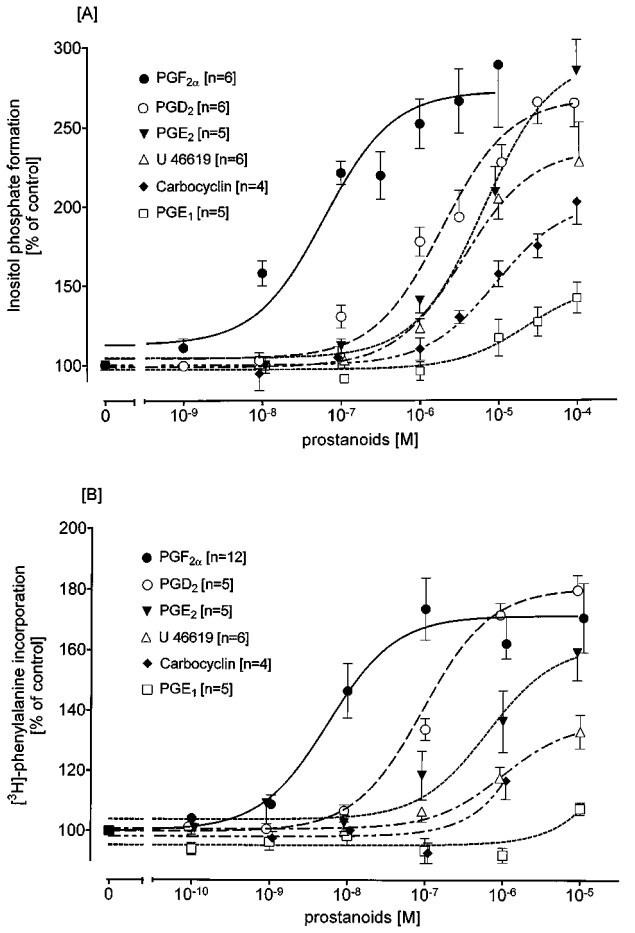

Figure 1 (A) Prostanoid-induced inositol phosphate (InsP) generation in neonatal rat ventricular cardiomyocytes. Ordinate scale: [^{3}H]-InsP-formation as per cent of basal formation. Abscissa scale: molar concentrations of prostanoids. Basal [^{3}H]-InsP-formation was 1–2% of the incorporated radioactivity and amounted to 1139 ± 144 c.p.m. in control cells (n = 22). (B) Prostanoid-induced [^{3}H]-phenylalanine incorporation in neonatal rat ventricular cardiomyocytes. Ordinate scale: [^{3}H]-phenylalanine incorporation as per cent of basal incorporation. Abscissa scale: molar concentrations of prostanoids. Basal [^{3}H]-phenylalanine incorporation in control cells was 3445 ± 185 c.p.m. (n = 18). In (A) and (B) values are means and vertical lines show s.e.mean.

formation by 50%, [S] = agonist concentration in the assay and EC_{50} = concentration of agonist inducing 50% of maximal InsP-formation (determined as described above). Statistical significance of differences were analysed by paired, two-tailed Student's *t*-test; a *P*-value <0.05 was considered significant. All statistical calculations were performed with the GraphPad Prism 2.01 programme.

Results

Effects of prostanoids on InsP-formation and rate of protein synthesis in neonatal rat cardiomyocytes

As discussed above, we had recently shown that in neonatal rat cardiomyocytes, the TXA_2-mimetic U 46619 weakly increased InsP-formation and rate of protein synthesis (assessed by [^{3}H]-phenylalanine incorporation), this was antagonized by the TP-receptor antagonist SQ 29548 with a potency (pK_B-value about 6) 10 – 100 time less than could be expected from the affinity of SQ 29548 for a TP-receptor (Coleman *et al*., 1994). We, therefore, studied the effects of several prostanoids on InsP-formation and rate of protein synthesis to find out whether another prostanoid receptor might be involved. All prostanoids studied ($PGF_{2\alpha}$, PGD_2, PGE_2, PGE_1, carbocyclin and U 46619) increased concentration dependently InsP-formation (Figure 1A) and [^{3}H]-phenylalanine incorporation (as a measure of rate of protein synthesis, Figure 1B). In both settings $PGF_{2\alpha}$ was about 100 times more potent than U 46619 (Figure 1, Table 1). Although prostanoid concentration-effect curves did not always yield clear maximum (Figure 1) and, hence, pEC_{50}-values could partly be only roughly calculated (Table 1) the order of potency for prostanoids induced increases in InsP-formation and rate of protein synthesis is best described as $PGF_{2\alpha} > PGD_2 \geqslant PGE_2 \geqslant$ U46619 $> PGE_1$.

Effects of prostanoids on InsP-formation and rate of protein synthesis in ventricular cardiomyocytes of adult rats

We next studied whether the characteristics of prostanoid-induced increases in InsP-formation and in rate of protein synthesis might be altered when cardiomyocytes were used that had been isolated from adult rats (12 weeks old). As shown in Figure 2, the order of potency for prostanoid-induced increases in InsP-formation and in rate of protein synthesis ($PGF_{2\alpha} > PGD_2 \geqslant PGE_2 >$ U 46619) was well comparable to that obtained in neonatal rat cardiomyocytes, (*c.f.* Figure 1). In addition, pEC_{50}-values for prostanoid-induced increases in InsP-formation and rate of protein synthesis (Table 2) were in a similar range as those obtained in neonatal rat cardiomyocytes. Moreover, as in the neonatal rat cardiomyocytes (Pönicke *et al*., 1999) also in the adult rat cardiomyocytes 10 μM SQ 29548 was only a very weak inhibitor of U 46619-induced increase in rate of protein synthesis (Figure 3). These data, therefore, strongly suggest that in adult rat cardiomyocytes the receptor mediating the effects of prostanoids is very similar to that in neonatal rat cardiomyocytes.

We also studied prostanoid-induced InsP-formation in left ventricular slices of rat heart. As shown in Figure 4, $PGF_{2\alpha}$ (10 nM – 100 μM) and U 46619 (10 nM – 100 μM) concentration-dependently increased InsP-formation; $PGF_{2\alpha}$ ($pEC_{50} = 6.6 \pm 0.2$) however, was about 100 times more potent than U 46619 ($pEC_{50} = 4.4 \pm 0.4$).

Effects of $PGF_{2\alpha}$ and U 46619 on force of contraction of isolated, electrically driven left ventricular strips of the rat heart

It is well known that in the heart of several species, stimulation of $G_{q/11}$-coupled receptors such as α_1-adrenergic or ET_A-receptors does not only lead to increases in InsP-formation but also to increases in force of contraction (Wagner & Brodde, 1978; Terzic *et al*., 1993; Rubanyi & Polokoff, 1994). We, therefore, studied whether $PGF_{2\alpha}$ or U 46619 might cause positive inotropic effects in the rat heart. As shown in Figure 5, $PGF_{2\alpha}$ (0.1 nM – 1 μM) caused a concentration-dependent increase in force of contraction of the isolated, electrically driven left ventricular strips; the pD_2-value (7.4 ± 0.1) was comparable with its pEC_{50}-value for increases in rate of protein synthesis in the adult rat cardiomyocytes (*c.f.* Table 2). Maximal increase was comparable with that induced by endothelin-1 (ET-1, $pD_2 = 8.0 \pm 0.1$) *via* ET_A-receptor stimulation, but less than that evoked by noradrenaline (in the presence of 1 μM propranolol, $pD_2 = 5.0 \pm 0.1$) *via* α_1-adrenoceptor stimulation. On the other hand, U 46619 (up to 1 μM) did not significantly affect basal force of contraction of the ventricular strips (Figure 5).

Effects of $PGF_{2\alpha}$ and U 46619 on InsP-formation and force of contraction of rat thoracic aorta

In a final set of experiments we assessed the effects of $PGF_{2\alpha}$ and U 46619 on InsP-formation and force of contraction of rat isolated thoracic aorta, a tissue known to contain a TP-receptor (Jones *et al*., 1989; Tymkewycz *et al*., 1991; Wagner *et al*., 1997). In rings of rat thoracic aorta U 46619 (10 nM – 10 μM) caused a pronounced increase in InsP-formation; maximal increase at 10 μM was 928 ± 189% of control (Figure 6A). The pEC_{50}-value for U 46619 was 6.9 ± 0.1 ($n = 4$). The TP-receptor antagonist SQ 29548 (1 nM – 10 μM) potently inhibited 1 μM U 46619-induced InsP-formation; pK_i-value was 8.2 ± 0.3 ($n = 3$), (Figure 6B). On the contrary, the effect of $PGF_{2\alpha}$ on InsP-formation in rat aortic rings was only weak,

Table 1 Effects of prostanoids on protein synthesis or InsP-formation in neonatal rat cardiomyocytes

	Protein-synthesis		*InsP-formation*	
	pEC_{50}	E_{max} (% of control)	pEC_{50}	E_{max} (% of control)
$PGF_{2\alpha}$	8.2 ± 0.2	171 ± 11	7.2 ± 0.3	289 ± 39
PGD_2	7.0 ± 0.1	180 ± 5	5.7 ± 0.1	265 ± 14
PGE_2	6.2 ± 0.2	160 ± 9	5.2 ± 0.2	285 ± 19
U 46619	6.0 ± 0.2	133 ± 5	5.5 ± 0.1	228 ± 35
Carbocyclin	n.d.	117 ± 6*	n.d.	202 ± 14
PGE_1	n.d.	108 ± 2*	n.d.	142 ± 10

$n = 4 – 12$ experiments, n.d. = no pEC_{50} could be determined. E_{max} = maximal increase in per cent of control (= 100%). *E_{max}-values for carbocyclin and PGE_1 do not represent maximum effects; given are effects at 1 μM (carbocyclin) and 10 μM (PGE_1).

Table 2 Effects of prostanoids on protein synthesis or InsP-formation in adult rat cardiomyocytes

	Protein-synthesis		*InsP-formation*	
	pEC_{50}	E_{max} (% of control)	pEC_{50}	E_{max} (% of control)
$PGF_{2\alpha}$	7.8±0.2	158±13	6.5±0.1	323±30
PGD_2	6.7±0.2	146±4	5.6±0.1	310±48
PGE_2	6.8±0.2	143±2	5.2±0.2	333±69
U 46619	6.2±0.3	128±8	4.6±0.1	151±6[a]

$n=4-6$ experiments. E_{max} = maximal increase in per cent of control (=100%). [a]E_{max}-value for U 46619 does not represent maximum effect; given are effects at 100 μM U 46619.

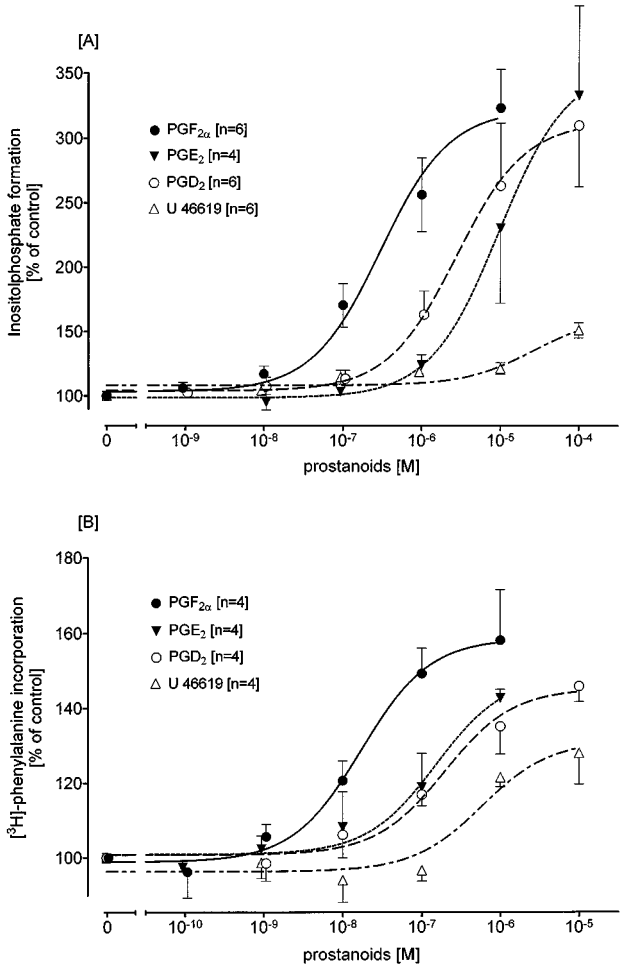

Figure 2 (A) Prostanoid-induced inositol phosphate (InsP) generation in adult male rat ventricular cardiomyocytes. Ordinate scale: [^{3}H]-InsP-formation as per cent of basal formation. Abscissa scale: molar concentrations of prostanoids. Basal [^{3}H]-InsP-formation was 1–2% of the incorporated radioactivity and amounted to 589±65 c.p.m. in control cells ($n=12$). (B) Prostanoid-induced [^{3}H]-phenylalanine incorporation in adult rat ventricular cardiomyocytes. Ordinate scale: [^{3}H]-phenylalanine incorporation as per cent of basal incorporation. Abscissa scale: molar concentrations of several prostanoids. Basal [^{3}H]-phenylalanine incorporation in control cells was 1845± 135 c.p.m. ($n=20$). In (A) and (B) values are means and vertical lines show s.e.mean.

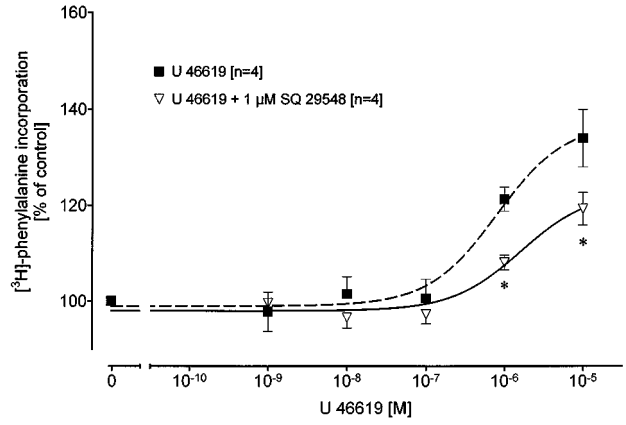

Figure 3 Effects of 1 μM SQ 29548 on U 46619-induced [^{3}H]-phenylalanine incorporation in adult male Wistar rat ventricular cardiomyocytes. Ordinate scale: [^{3}H]-phenylalanine incorporation as per cent of basal incorporation. Abscissa scale: molar concentrations of U 46619. Basal [^{3}H]-phenylalanine incorporation in control cells was 1445±111 c.p.m. ($n=4$); values are means and vertical lines show s.e.mean. *$P<0.05$ vs U 46619.

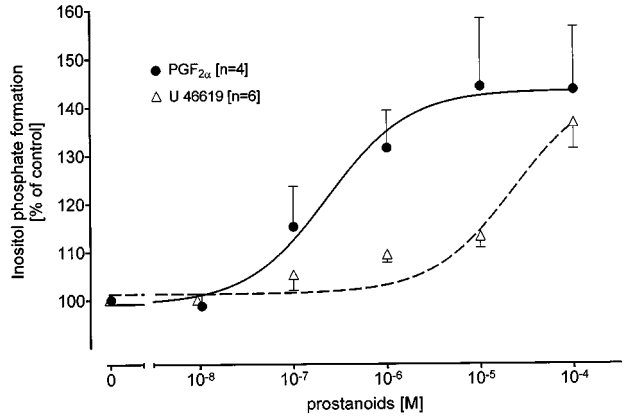

Figure 4 $PGF_{2\alpha}$ or U 46619-induced inositol phosphate (InsP) generation in left ventricular slices of 12 week old male Wistar rats. Ordinate scale: [^{3}H]-InsP-formation as per cent of basal formation. Abscissa scale: molar concentrations of U 46619 or $PGF_{2\alpha}$. Basal [^{3}H]-InsP-formation was about 0.25% of the incorporated radioactivity and amounted to 92±8 c.p.m. in control ventricular slices ($n=10$); values are means and vertical lines show s.e.mean.

maximal increase at 100 μM was 254±28% of control ($n=5$). It should be noted, that $PGF_{2\alpha}$-curve fitted significantly better to a two-side model than to a one-side model (*F*-test: $P<0.01$); at lower concentrations (up to 1 μM) it increased InsP-formation by only 50%, while the second component started at concentration >1 μM. Ten μM $PGF_{2\alpha}$-evoked InsP-formation was inhibited by SQ 29548 with an IC_{50}-value of 17.1±2 nM ($n=5$) (Figure 6B).

Finally, we studied the effects of U 46619, $PGF_{2\alpha}$ and PGD_2 on developed tension in isolated helically cut strips of rat thoracic aorta. All three agonists led to a concentration-dependent contraction of rat aorta. However, U 46619 (pD_2-value 8.1±0.1, $n=17$) was about 100 times more potent than $PGF_{2\alpha}$ or PGD_2. Again, concentration-response curve for

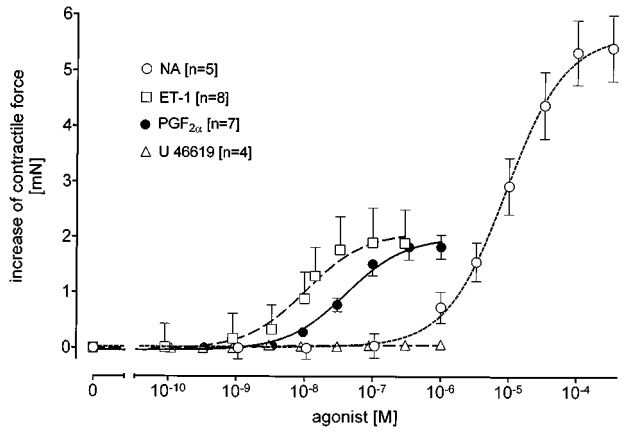

Figure 5 Effects of $PGF_{2\alpha}$, U 46619, noradrenaline (NA) or endothelin (ET)-1 on contractile force of isolated electrically driven left ventricular strips of 12 week old male Wistar rats. Ordinate scale: increase of contractile force in mN; abscissa scale: molar concentrations of the agonists. Basal force of contraction was 2.75 ± 0.2 mN ($n=5$) in the NA-experiments, 3.04 ± 0.2 mN ($n=8$) in the ET-1-experiments, 3.4 ± 0.3 mN ($n=7$) in the $PGF_{2\alpha}$-experiments and 2.56 ± 0.3 mN ($n=4$) in the U 46619-experiments; values are means and vertical lines show s.e.mean.

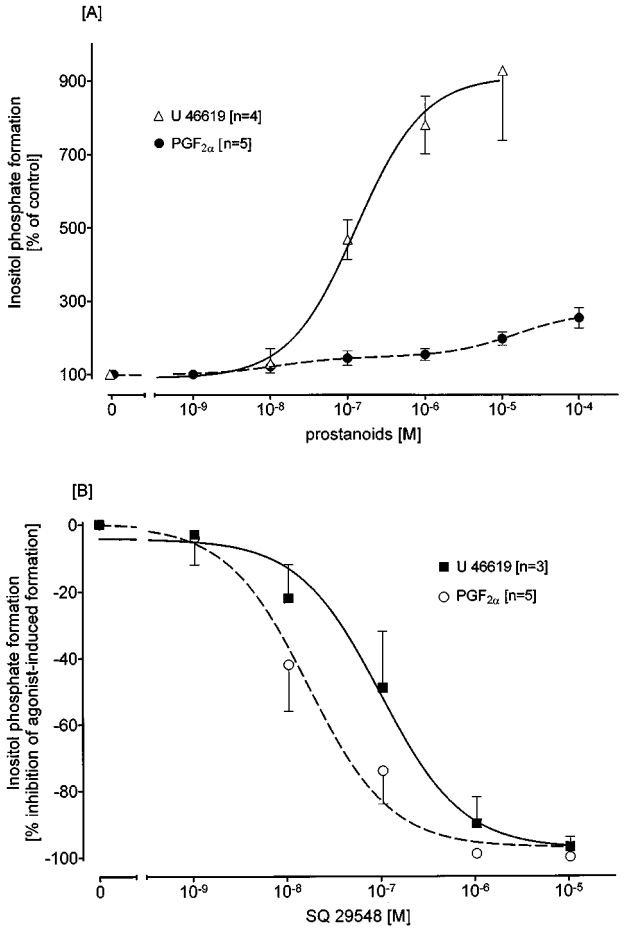

Figure 6 (A) $PGF_{2\alpha}$ or U 46619-induced inositol phosphate (InsP) generation in rings of thoracic aorta of 12 week old Wistar rats. Ordinate scale: [^{3}H]-InsP-formation as per cent of basal formation. Abscissa scale: molar concentrations of $PGF_{2\alpha}$ or U 46619. Basal [^{3}H]-InsP-formation was 0.5–1.0% of the incorporated radioactivity and amounted to 326 ± 38 c.p.m. in control aortic rings ($n=9$). (B) Effect of the TP-receptor antagonist SQ 29548 on 1 μM U 46619 or 10 μM $PGF_{2\alpha}$-induced inositol phosphate (InsP) generation in rings of thoracic aorta of 12 week old male Wistar rats. Ordinate scale: per cent inhibition of agonist-induced [^{3}H]-InsP-formation. Abscissa scale: molar concentrations of SQ 29548. [^{3}H]-InsP-formation was after 1 μM U 46619 1256 ± 380 c.p.m. ($n=3$) and after 10 μM $PGF_{2\alpha}$ 366 ± 62 c.p.m. ($n=5$). In (A) and (B) values are means and vertical lines show s.e.mean.

$PGF_{2\alpha}$ fitted significantly better to a two-side model than to a one-side model (F-test: $P<0.001$) (Figure 7A).

The TP-receptor antagonist SQ 29548 (10 nM) significantly shifted the concentration-response curve for U 46619 to the right (Figure 7B); the pK_B-value was estimated to 8.8 ± 0.2 ($n=10$). Similarly, SQ 29548 caused an about 10 fold shift of the concentration-response curve for $PGF_{2\alpha}$ to the right (Figure 7C); moreover, in the presence of SQ 29548, $PGF_{2\alpha}$ concentration-response curve was monophasic (Figure 7C).

Discussion

In the present study the effects of several prostanoids on InsP-formation and rate of protein synthesis (assessed as [^{3}H]-phenylalanine incorporation) were assessed in ventricular cardiomyocytes from neonatal as well as adult rats. In both preparations $PGF_{2\alpha}$ was the most potent prostanoid in inducing increases in InsP-formation and in rate of protein synthesis whereas the TP-receptor agonist U 46619 was only a weak agonist causing effects only in concentrations >1 μM. Similarly, in slices of the left ventricle of the adult rat heart $PGF_{2\alpha}$ was about 100 times more potent than U 46619 in increasing InsP-formation. For all prostanoids studied the order of potency for increasing InsP-formation and rate of protein synthesis was in neonatal ($PGF_{2\alpha} > PGD_2 \geqslant PGE_2 \geqslant$ U 46619 $> PGE_1$) well comparable with that in adult rat cardiomyocytes ($PGF_{2\alpha} > PGD_2 \geqslant PGE_2 >$ U 46619).

This order of potency was in marked contrast to that obtained for prostanoid-effects in rat thoracic aorta, a tissue widely used to study TP-receptor-mediated effects (Jones *et al*., 1989; Tymkewycz *et al*., 1991; Wagner *et al*., 1997). Thus, in slices of rat thoracic aorta U 46619 was much more potent than $PGF_{2\alpha}$ in increasing InsP-formation; maximal increases induced by U 46619 were obtained in concentrations between 1–10 μM, i.e. concentrations where U 46619 just started to evoke effects in the cardiomyocytes. The same held true for prostanoid-induced contractions of the isolated helically cut strip of the thoracic aorta. U 46619 evoked contractions with a potency about 100 times greater than that of $PGF_{2\alpha}$ or PGD_2. Moreover, U 46619- as well as $PGF_{2\alpha}$-effects were inhibited in thoracic aorta by the selective TP-receptor antagonist SQ 29548 with a potency (pK_B-values of about 8.0–9.0) that was well in the range of its affinity at the TP-receptor (Coleman *et al*., 1994). On the other hand, SQ 29548, even in the high concentration of 1 μM, did only marginally affect U 46619-evoked increase in protein synthesis in neonatal (Pönicke *et al*., 1999) and adult cardiomyocytes (*c.f.* Figure 3). Thus, the order of potency U 46619 $\gg$ $PGF_{2\alpha} = PGD_2$ and the high antagonistic potency of SQ 29548 confirm that prostanoid-induced effects in rat thoracic aorta are mediated by a TP-receptor. Accordingly, the present results clearly demonstrate that the prostanoid receptor subtype mediating the hypertrophic response in rat cardiomyocytes is not a TP-receptor; the order of potency for prostanoid-induced effects ($PGF_{2\alpha} > PGD_2 \geqslant PGE_2 >$ U 46619) strongly supports the view that increases in InsP-formation and in rate of protein synthesis are mediated predominantly, if not exclusively, by an FP-receptor (Coleman *et al*., 1994). It should be noted that this holds true not only for neonatal rat cardiomyocytes (in agreement with data from the literature, Adams *et al*., 1996;

Lai *et al*., 1996; Kunapuli *et al*., 1998) but also for cardiomyocytes isolated from left ventricle of the adult rat heart. Thus, these results indicate that (at least for prostanoid mediated effects) data obtained in neonatal cardiomyocytes are comparable with those obtained in adult cardiomyocytes. It has been recently shown that, in sarcolemmal membranes of pig heart an E-type prostaglandin-receptor (EP_3) exists that is completely different from the FP-receptor in rat cardiomyocytes: this EP_3-receptor couples *via* a pertussis toxin sensitive G-protein (G_i) to adenylyl cyclase in an inhibitory fashion and exerts antiadrenergic effects (Hohlfeld *et al*., 1997).

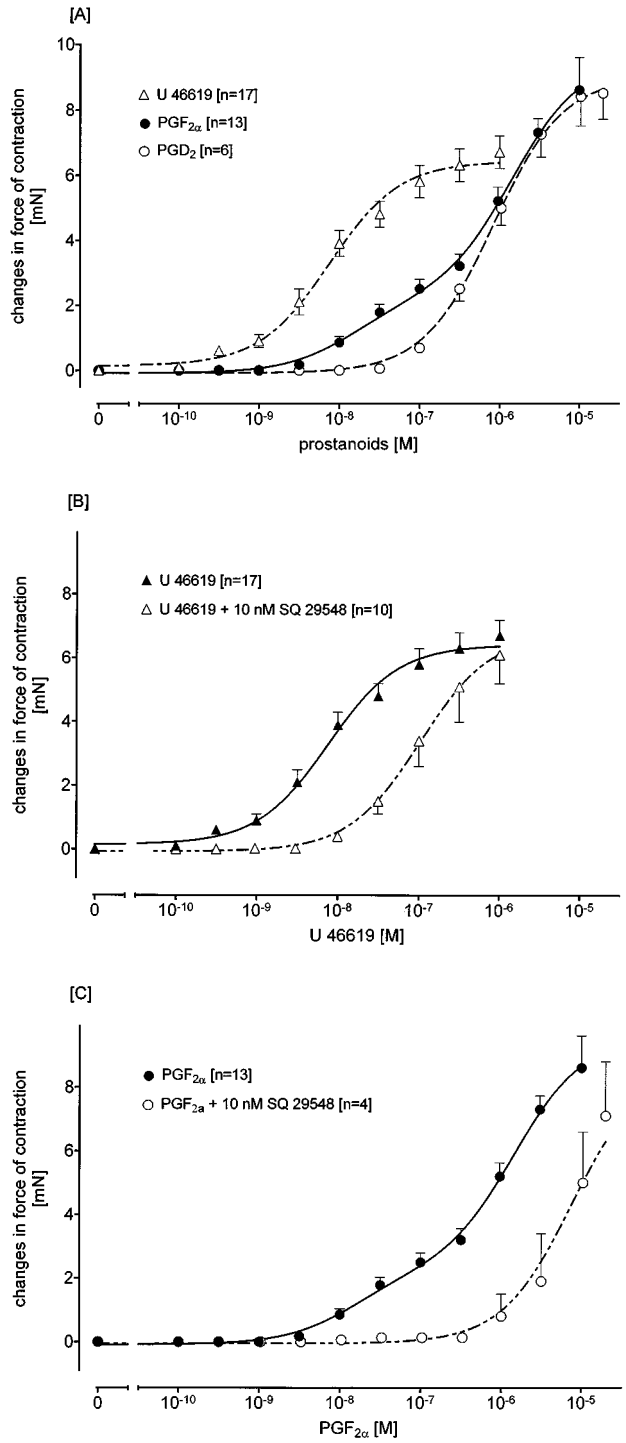

Figure 7 (A) Effects of U 46619, $PGF_{2\alpha}$ or PGD_2 on contractile force of helical strips of isolated thoracic aorta of 12 weeks old male Wistar rats. Ordinate scale: changes in force of contraction in mN. Abscissa scale: molar concentrations of U 46619, $PGF_{2\alpha}$ or PGD_2. (B) Effect of the TP-receptor antagonist SQ 29548 (10 nM) on U 46619-evoked changes of contractile force of helical strips of rat isolated thoracic aorta. Ordinate scale: changes in mN. Abscissa scale: molar concentrations of U 46619. (C) Effect of the TP-receptor antagonist SQ 29548 (10 nM) on $PGF_{2\alpha}$-evoked changes of contractile force of helical strips of rat isolated thoracic aorta. Ordinate scale: changes in contractile force in mN. Abscissa scale: molar concentrations of $PGF_{2\alpha}$. In (A), (B) and (C) values are means and vertical lines show s.e.mean.

Hoffmann *et al*. (1993) and Dogan *et al*. (1997) have shown that, in neonatal rat cardiomyocytes, U 46619 induced increases in Ca^{2+} transients, and this could be inhibited by the TP-receptor antagonists SK&F95585 (2 μM, Hoffmann *et al*., 1993) and SQ 29548 (10 μM, Dogan *et al*., 1997). Thus, it had been proposed that these effects of U 46619 are mediated by a TP receptor. However, these data are not necessarily contradictory to the present results. In both reports U 46619 caused significant effects in concentrations $\geqslant$100 nM, a concentration that was also in the present study threshold concentration for U 46619-induced InsP-formation and in rate of protein synthesis (*c.f*. Figure 1) in the cardiomyocytes. On the other hand, in the classical TP-receptor system, the thoracic aorta, at this concentration (100 nM) U 46619 caused nearly maximal contraction (*c.f*. Figure 7). Moreover, concentrations for antagonists used (2 μM for SK&F 95587, i.e. 5–10 times K_I (Tymkewycz *et al*., 1991); 10 μM for SQ 29548, i.e. 100–1000 times K_I (Ogletree *et al*., 1985)) were rather high so that nonspecific effects can not be excluded. It is therefore, well possible that the effects of U 46619 on Ca^{2+}-transients are not mediated by a TP-receptor but (according to the present results) by an FP-receptor.

It has been shown that in the heart of several species, including the rat, stimulation of $G_{q/11}$-coupled receptors can lead to increases in contractile force. Thus, many studies have demonstrated that in rat heart stimulation of α_1-adrenoceptors (Wagner & Brodde, 1978; Terzic *et al*., 1993) or ET_A-receptors (Rubanyi & Polokoff, 1994) evoked increases in force of contraction on left ventricular preparations. The same holds true also for the FP-receptor: as shown in Figure 4 $PGF_{2\alpha}$ (that causes its effects on the cardiomyocytes *via* the FP-receptor, see above), concentration-dependently increased force of contraction of the isolated electrically driven left ventricular strip of the rat heart. Its pD_2-value (7.4) was in good agreement with its pEC_{50}-value (7.8, see Table 2) for increasing rate of protein synthesis in the adult cardiomyocytes. Maximal increases in contractile force evoked by $PGF_{2\alpha}$ were comparable with those induced by ET-1 (*via* ET_A-receptors) but less than those evoked by noradrenaline (*via* α_1-adrenoceptors). On the other hand, U 46619 (up to 1 μM) did not affect basal force of contraction of the left ventricular strips–in contrast to its effect on the isolated helically cut strips of the thoracic aorta where the 1 μM concentration caused nearly maximal contractile effects (*c.f*. Figure 7). Thus, also for inducing positive inotropic effects the order of potency $PGF_{2\alpha} \gg$ U 46619 indicates involvement of an FP-receptor.

The mechanism underlying positive inotropic effects evoked by $G_{q/11}$-coupled receptors is not completely understood. Activation of these receptors causes formation of $InsP_3$ and DAG with the former mediating the release of Ca^{2+} from intracellular stores which might be involved in increases of force of contraction. In addition, however, it has been shown that stimulation of α_1-adrenoceptors (for review see Terzic *et al*., 1993) and ET_A-receptors (Krämer *et al*., 1991; Meyer *et al*., 1996) increases the Ca^{2+}-sensitivity of myofilaments *via* activation of the Na^+/H^+-antiporter and it has been suggested that these effects are (at least partly) due to DAG-induced activation of PKC. It is reasonable to assume that the positive

inotropic effect of $PGF_{2\alpha}$ (*via* the $G_{q/11}$-coupled FP-receptor) is brought about by a similar mechanism as that for α_1- and ET_A-receptors. In fact, Yew *et al*. (1998) have recently shown, that in isolated rat ventricular cardiomyocytes $PGF_{2\alpha}$ increased single myocyte shortening and reduced resting cell-length in a concentration-dependent manner. This positive inotropic action was inhibited by the Na^+/H^+-antiporter inhibitor HOE 694 and by the PKC-inhibitor chelerythrine. Thus, the positive inotropic effect of $PGF_{2\alpha}$ appears to be mediated *via* activation of the Na^+/H^+-antiporter with the possible involvement of PKC. That would also explain why U 46619 up to 1 μM does not affect force of contraction in the ventricular strips: at this concentration U 46619 does neither in adult cardiomyocytes nor in left ventricular slices stimulate PLC (as demonstrated by the lack of effect of U 46619 on InsP-formation).

In conclusion: In neonatal as well as adult rat ventricular cardiomyocytes prostanoids can increase inositol phosphate formation and rate of protein synthesis; these growth-promoting effects of prostanoids are mediated by activation of an FP-receptor. Studies are now in progress to investigate whether or not prostanoids play a pathophysiologic role in development and/or maintenance of cardiac hypertrophy.

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References

ADAMS, J.W., MIGITA, D.S., YU, M.K., YOUNG, R., HELLICKSON, M.S., CASTRO-VARGAS, F.E., DOMINGO, J.D., LEE, P.H., BUI, J.S. & HENDERSON, S.A. (1996). Prostaglandin $F_{2\alpha}$ stimulates hypertrophic growth of cultured neonatal rat ventricular myocytes. *J. Biol. Chem.,* **271,** 1179–1186.

CHENG, Y.-C. & PRUSOFF, W.H. (1973). Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.,* **22,** 3099–3108

COLEMAN, R.A., SMITH, W.L. & NARUMIYA, S. (1994). VIII. International Union of Pharmacology. Classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes. *Pharmacol. Rev.,* **46,** 205–229.

DOGAN, S., TURNBAUGH, D., ZHANG, M., COFIE, D.Q., FUGATE, R.D. & KEM, D.D. (1997). Thromboxane A_2 receptor mediation of calcium transient in rat cardiomyocytes. *Life Sciences,* **60,** 943–952.

DORN, G.W. II & BROWN, J.H. (1999). G_q signaling in cardiac adaptation and maladaptation. *Trends Cardiovasc. Med.,* **9,** 26–34.

HOFFMAN, P., HEINROTH-HOFFMANN, I. & TORAASON, M. (1993). Alteration by a thromboxane A_2 analog (U46619) of calcium dynamics in isolated rat cardiomyocytes. *J. Pharmacol. Exp. Ther.,* **264,** 336–344.

HOHLFELD, T., ZUCKER, T.-P., MEYER, J. & SCHRÖR, K. (1997). Expression, function, and regulation of E-type prostaglandin receptors (EP_3) in the nonischemic and ischemic pig heart. *Circ. Res.,* **81,** 765–773.

ITO, H., HIRATA, Y., ADACHI, S., TANAKA, M., TSUJINO, M., KOIKE, A., NOGAMI, A., MURUMO, F. & HIROE, M. (1993). Endothelin-1 is an autocrine/paracrine factor in the mechanism of angiotensin II-induced hypertrophy in cultured rat cardiomyocytes. *J. Clin. Invest.,* **92,** 398–403.

JONES, R.L., WILSON, N.H. & LAWRENCE, R.A. (1989). EP 171: a high affinity thromboxane A_2-mimetic, the action of which are slowly reversed by receptor blockade. *Br. J. Pharmacol.,* **96,** 875–887.

KNOWLTON, K.U., MICHEL, M.C., ITANI, M., SHUBEITA, H.E., ISHIKARA, K., BROWN, J.H. & CHIEN, K.R. (1993). The α_{1A}-adrenergic receptor subtype mediates biochemical, molecular, and morphologic features of cultured myocardial cell hypertrophy. *J. Biol. Chem.,* **268,** 15374–15380.

KOTCHI KOTCHI E., WEISSELBERG, T., RÖHNERT, P., PREISS, M., HEINROTH-HOFFMAN, I., OSTEN, B., BRODDE, O.-E. (1998). Nitric oxide inhibits isoprenaline-induced positive inotropic effects in normal, but not in hypertrophied rat heart. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **357,** 579–583.

KRÄMER, B.K., SMITH, T.W. & KELLY, R.A. (1991). Endothelin and increased contractility in adult rat ventricular myocytes. Role of intracellular alkalosis induced by activation of the protein kinase C-dependent Na^+/H^+ exchanger. *Circ. Res.,* **68,** 269–279.

KUNAPULI, P., LAWSON, J.A., ROKACH, J.A., MEINKOTH, J.L. & FITZGERALD, G.A. (1998). Prostaglandin F_{2a} and the isoprostane, 8, 12-iso-isoprostane $F_{2\alpha}$-III, induce cardiomyocyte hypertrophy. Differential activation of downstream signaling pathways. *J. Biol. Chem.,* **272,** 22442–22452.

LAI, J., JIN, H., YANG, R., WINER, J., LI, W., YEN, R., KING, K.L, ZEIGLER, F., KO, A., CHENG, J., BUNTING, S. & PAONI, N.F. (1996). Prostaglandin $F_{2\alpha}$ induces cardiac myocyte hypertrophy in vitro and cardiac growth in vivo. *Am. J. Physiol.,* **271**, H2197–H2208.

LEE, H.R., HENDERSON, S.A., REYNOLDS, R., DUNNMON, P., YUAN, D. & CHIEN, K.R. (1988). α_1-Adrenergic stimulation of cardiac gene transcription in neonatal rat myocardial cells: effects on myosin light chain-2 gene expression. *J. Biol. Chem.,* **263,** 7352–7358.

MEYER, M., LEHNART, S., PIESKE, B., SCHLOTTAUER, K., MUNK, S., HOLUBARSCH, C., JUST, H. & HASENFUSS, G. (1996). Influence of endothelin-1 on human atrial myocardium - myocardial function and subcellular pathways. *Basic Res. Cardiol.,* **91,** 86–93.

OGLETREE, M.L., HARRIS, D.N., GREENBERG, R., HASLANGER, M.F. & NAKANE, M. (1985). Pharmacological action of SQ 29,548, a novel selective thromboxane antagonist. *J. Pharmacol. Exp. Ther.,* **234,** 435–441.

PIPER, H.M. & VOLZ, A. (1990). Adult ventricular rat heart muscle cells. In: Piper HM (ed). *Cell culture techniques in heart and vessel research*. Springer-Verlag, Heidelberg Germany, pp. 158–177.

PÖNICKE, K., HEINROTH-HOFFMANN, I., BECKER, K. & BRODDE, O.-E. (1997). Trophic effect of angiotensin II in neonatal rat cardiomyocytes: role of endothelin-1 and non-myocyte cells. *Br. J. Pharmacol.,* **121:** 118–124.

PÖNICKE, K., HEINROTH-HOFFMANN, I., BECKER, K., OSTEN, B. & BRODDE, O.-E. (1999). $G_{q/11}$ coupled receptors and protein synthesis in rat cardiomyocytes: role of G_i-proteins and protein kinase C-isozymes. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **360,** 301–308.

PÖNICKE, K., VOGELSANG, M., HEINROTH, M., BECKER, K., ZOLK, O., BÖHM, M., ZERKOWSKI, H.R. & BRODDE, O.-E. (1998). Endothelin receptors in the failing and nonfailing human heart. *Circulation,* **97,** 744–751.

RUBANYI, G.M. & POLOKOFF, M.A. (1994). Endothelins: molecular biology, biochemistry, pharmacology, physiology, and pathophysiology. *Pharmacol. Rev.,* **46,** 325–415.

SHUBEITA, H.E., MCDONOUGH, P.M., HARRIS, A.N., KNOWLTON, K.U., GLEMBOTZKI, C.C., BROWN, J.H. & CHIEN, K.R. (1990). Endothelin induction of inositol phospholipid hydrolysis, sarcomere assembly, and cardiac gene expression in ventricular myocytes. A paracrine mechanism for myocardial cell hypertrophy. *J. Biol. Chem.,* **265,** 20555–20562.

SIMPSON, P. (1983). Norepinephrine-stimulated hypertrophy of cultured rat myocardial cells is an α-adrenergic response. *J. Clin. Invest.,* **72,** 732–738.

SUGDEN, P.H. & BOGOYEVITCH, M.A. (1995). Intracellular signalling through protein kinases in the heart. *Cardiovasc. Res.,* **30,** 478–492.

SUGDEN, P.H. & CLERK, A. (1998). Cellular mechanisms of cardiac hypertrophy. *J. Mol. Med.,* **76,** 725–746.

SUGDEN, P.H., FULLER, S.J., MYNETT, J.R., HATCHETT, R.J., BOGOYEVITCH, M.A. & SUGDEN, M.C. (1993). Stimulation of adult rat ventricular myocyte protein synthesis and phosphoinoisitide hydrolysis by the endothelins. *Biochim. Biophys. Acta,* **1175,** 327 – 332.

SUZUKI, T., HOSHI, H. & MITSUI, Y. (1990). Endothelin stimulates hypertrophy and contractility of neonatal rat cardiac myocytes in a serum-free medium. *FEBS Lett.,* **268,** 149 – 151.

TERZIC, A., PUCEAT, M., VASSORT, G. & VOGEL, SM. (1993). Cardiac alpha 1-adrenoceptors: an overview. *Pharmacol. Rev.,* **45,** 147 – 175.

TYMKEWYCZ, P.M., JONES, R.L., WILSON, N.H. & MARR C.G. (1991). Heterogeneity of thromboxane A_2 (TP-) receptors: evidence from antagonist but not agonist potency measurements. *Br. J. Pharmacol.,* **102,** 607 – 614.

VIKO, H., OSNES, J.-B., SJETNAN, A.E. & SKOMEDAL, T. (1995). Improved isolation of cardiomyocytes by trypsination in addition to collagenase treatment. *Pharmacol. & Toxicol.,* **76,** 68 – 71.

WAGNER, J. & BRODDE, O.-E. (1978). On the presence and distribution of alpha-adrenoceptors in the heart of various mammalian species. *Naunyn-Schmiedebergs Arch. Pharmacol.,* **302,** 239 – 254.

WAGNER, R.S., WEARE, C., JIN, N., MOHLER, E.R. & RHOADES, R.A. (1997). Characterization of signal transduction events stimulated by 8-epi-prostaglandin(PG)$F_{2\alpha}$ in rat aortic rings. *Prostaglandins,* **54,** 581 – 599.

YEW, S.F., REEVES, K.A. & WOODWARD, B. (1998). Effects of prostaglandin F_{2a} on intracellular pH, intracellular calcium, cell shortening and L-type calcium currents in rat myocytes. *Cardiovasc. Res.,* **40,** 538 – 545.

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