The platelet-derived growth factor isomers, PDGF-AA, PDGF-AB and PDGF-BB, induce contraction of vascular smooth muscle cells by different intracellular mechanisms

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The effect of human recombinant platelet-derived growth factor (PDGF) isoforms, (r)PDGF-AA, PDGF-AB and PDGF-BB, on contractility of rat aortic rings as well as on intracellular free Ca^{2+} ($[Ca^{2+}]_i$), intracellular pH, (pH_i) and thromboxane A_2 (TXA_2) formation in cultured vascular smooth muscle cells (VSMC) was examined. PDGF-BB behaved similar to PDGF-AB and both have features characteristic of conventional vasoconstrictor-agonists that directly increase $[Ca^{2+}]_i$, activate the Na^+/H^+ exchanger, stimulate the TXA_2 formation, and induced contraction in VSMC whereas PDGF-AA induced contraction without increasing of $[Ca^{2+}]_i$, pH_i, and TXA_2 formation.

PDGF-isoform; Vasoconstriction; Intracellular Ca^{2+} and pH; Thromboxane A_2

1. INTRODUCTION

PDGF is a potent mitogen in vitro for fibroblasts, glial cells and VSMC [1,2] and thus plays an important role in the pathogenesis of atherosclerosis [3]. In addition, it has been shown that PDGF is a potent vasoconstrictor of rat aortic tissue [4]. PDGF consists of two homologous chains, termed A and B, which are stabilized by a disulfide bridge. The two chains can combine to three different dimeric isoforms, PDGF-AA, PDGF-AB and PDGF-BB [1,2,5]. It is generally accepted that agonist-mediated increases in cytosolic calcium in VSMC and intracellular pH (pH_i) acidification result in a contractile response in VSMC [6,7]. TXA_2 is also a potent vasoconstrictor of VSMC as well as a platelet aggregatory agent [8,9].

Here the effect of human recombinant (r)PDGF-AA, PDGF-AB and PDGF-BB on contractility of aortic rings was investigated. In addition the effect of the three isoforms on $[Ca^{2+}]_i$, pH_i and TXA_2 formation was examined.

2. MATERIALS AND METHODS

2.1. *Materials*

rPDGF-AA, rPDGF-AB, and rPDGF-BB from *E. coli* were prepared as described [5]. Fura-2/AM, pentaacetoxymethyl ester was obtained from Calbiochem. DMEM, FCS and PBS were obtained from Gibco. TXB_2-RIA kit was obtained from New England Nuclear. Other chemicals were from Sigma and Merck-Schuchardt.

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Abbreviations: $[Ca^{2+}]_i$, intracellular free Ca^{2+}; pH_i, intracellular pH; TXA_2, thromboxane A_2; VSMC, vascular smooth muscle cells; PDGF, platelet-derived growth factor; BCECF, 2',7'-bis(carboxyethyl)-5,6-carboxyfluorescein; PBS, phosphate-buffered saline; Hepes, 4-(2-hydroxyethyl)piperazine-1-ethane-sulfonic acid

2.2. *Culture of vascular smooth muscle cells*

Vascular smooth muscle cells were isolated from rat aorta (female, strain Wistar-Kyoto, 6-8 weeks old) and cultured over several passages according to Ross [10]. The cells were allowed to grow for 4-5 days in 5% CO_2, 95% air at 37°C. The culture medium was Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum.

2.3. *Aortic ring contractile responses*

These measurements were performed according to Marriot et al. [11]. 3 mm long rings of thoracic aorta from female Wistar-Kyoto rats (300 g) were mounted under resting tension of 3.5 g in a Krebs solution of the following composition (in mM): NaCl 118.4, KCl 4.75, $CaCl_2$ 2.5, $MgSO_4 \cdot 7H_2O$ 1.18, KH_2PO_4 1.19, $NaHCO_3$ 25, glucose 11.66, ascorbic acid 0.05, and EDTA 0.01, maintained at 37°C and gassed with 5% CO_2 in O_2.

The rings were equilibrated for 1 h before experimentation and during this period the bathing fluid was changed every 20 min. Check of endothelium was assessed by vasodilatation of rings, which were contracted with 100 nM norepinephrine. The contractile response of the rings was measured isotonically with a transducer (Lever transducer type B 368, Hugo Sachs Electronics) after the administration of the rPDGF-isoforms.

2.4. *Measurement of $[Ca^{2+}]_i$ and pH_i*

Confluent cells were detached with 0.04% trypsin/0.02% EGTA/Puck's Saline A physiological solution after 5-10 min at 37°C. The cells were cultured on glass microscope slides (26 × 76 mm) under normal tissue culture conditions. When the cells became confluent (20 000–40 000 cells/cm^2) cells were incubated with 2 μM fura-2 pentaacetoxymethyl ester at 37°C for 20 min in Hepes-buffer (20 mM Hepes, 16 mM glucose, 130 mM NaCl, 1 mM $MgSO_4 \cdot 7H_2O$, 0.5 mM $CaCl_2$, Tris-base, pH 7.4) supplemented with 1% bovine serum albumin (BSA). Just prior to the measurements, the cell monolayers were rinsed with Hepes buffer, containing 1 mM $CaCl_2$

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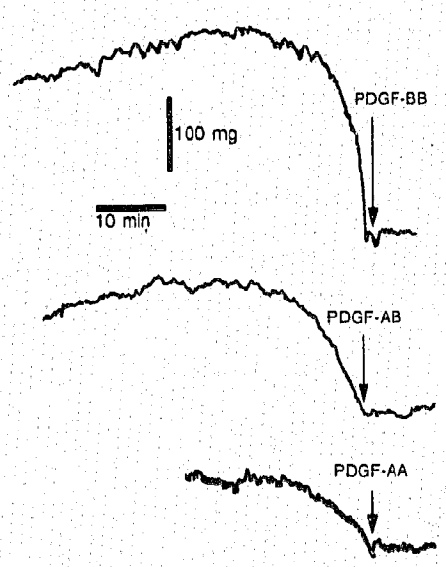

Fig. 1. PDGF-isoforms stimulated contractile responses of rat aorta rings. Contractile response of rat aorta rings in presence of 2.5 mM $CaCl_2$ to 10 ng/ml PDGF-BB (upper trace), to 10 ng/ml PDGF-AB (middle trace) and to 10 ng/ml PDGF-AA (lower trace).

and the glass slide was positioned diagonally in the cuvette. The Ca^{2+}-fura-2 fluorescence was measured at 37°C under stirring in a SLM-Aminco SPF-500 spectrofluorometer (excitation wavelengths 340 and 380 nm; emission: 505 nm). Fluorescence signals were calibrated according to Grynkiewicz et al. [12]. The measurements of pH_i in VSMC were performed according to Berk et al. [13] with the fluorescence pH indicator [2',7'-bis(carboxyethyl)-5,6-carboxyfluorescein] (BCECF). Cell monolayers were loaded as described for the Fura-2 loading method in Hepes-buffer with 4 μM BCECF-pentaacetoxymethyl ester for 20 min at 37°C. For the fluorescence measurements the following wavelengths were set - excitation wavelengths 500 and 455 nm; emission wavelength 525 nm. The calibration curve was performed by permeabilizing the cells with 0.7 μg/ml nigericin as previously described [13].

2.3. *Measurement of TXA_2 formation in VSMC*

VSMC were cultured in 60 mm diameter Petri dishes. After 3 days, cells reached confluence. Cells were washed twice with Hepes-buffer and stimulated for various time intervals with 10 ng/ml PDGF-isoforms. The reaction was terminated by freezing the cell layers in a dry-ice/methanol bath. TXB_2 purification was carried out according to the instruction of the TXB_2-RIA kit (New England Nuclear). TXA_2 was determined as TXB_2 with radioimmunological methods, since TXA_2 is not stable and rapidly hydrolized to TXB_2 (Introduction RIA kit, New England Nuclear).

3. RESULTS

Fig. 1 shows a representative experiment of six individual experiments. Addition of 10 ng/ml PDGF-BB, PDGF-AB and PDGF-AA produced a contractile tension of 145 ± 28 mg (100%) (mean ± SD), 122 ± 25 mg (84%), 58 ± 14 mg (40%), respectively. Hence it follows that PDGF-BB had a greater efficacy than PDGF-AB and PDGF-AA.

As depicted in Fig. 2, PDGF-BB and PDGF-AB induced a rapid rise in $[Ca^{2+}]_i$ (basal value = 85 ± 11 nM, mean ± SD, $n = 24$) with a maximum of 211 ± 14

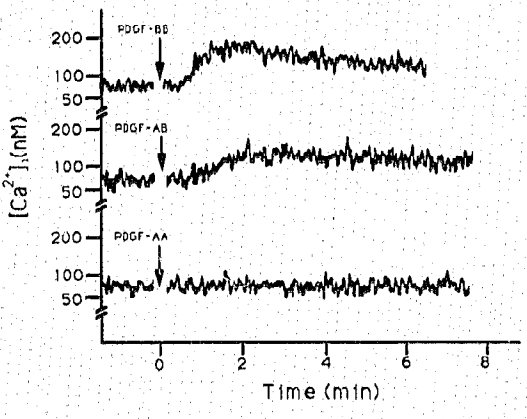

Fig. 2. Effect of PDGF-isoforms on $[Ca^{2+}]_i$ in cultured rat VSMC monolayers. Effect of 10 ng/ml PDGF-BB (upper trace), 10 ng/ml PDGF-AB (middle trace), 10 ng/ml PDGF-AA (lower trace). $[Ca^{2+}]_i$ was measured by the Fura-2 method as described in section 2. Arrows indicate addition of PDGF-isoforms.

nM and 190 ± 12 nM ($n = 5$) at 25 and 45 s, respectively. PDGF-AA (1–1000 nM) did not rise $[Ca^{2+}]_i$. The PDGF-BB- and -AB-induced rise of $[Ca^{2+}]_i$ declined after 25 and 45 s towards the resting level within 3 min.

The resting pH of VSMC was 7.15 ($n = 12$). The effects of PDGF-isoforms on the pH_i are shown in Fig. 3. After addition of 10 ng/ml PDGF-BB to the cells, initially a small acidification of about 0.05 pH units occurred followed by an alkalinization with return to basal pH_i within 2.8 min. Changes of pH_i induced by 10 ng/ml PDGF-AB were similar but occurred more slowly than those with PDGF-BB. 1–1000 ng/ml PDGF-AA did not cause any changes of pH_i in VSMC. Again a lack of a response in pH_i upon incubation of VSMC with 10 ng/ml reduced PDGF-BB and -AB was observed.

Fig. 4 shows one representative experiment of the TXA_2 formation after stimulation of VSMC for

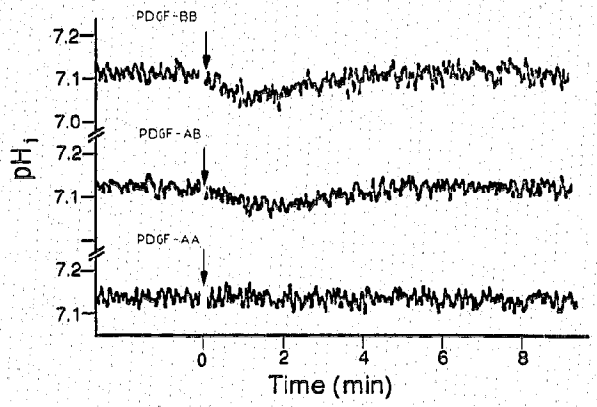

Fig. 3. Effect of PDGF-isoforms on pH_i in cultured rat VSMC. Effect of 10 ng/ml PDGF-BB (upper trace), 10 ng/ml PDGF-AB (middle trace), and 10 ng/ml PDGF-AA (lower trace). VSMC were prepared and loaded with BCECF as described in section 2. Arrows indicate addition of the PDGF-isomorphs.

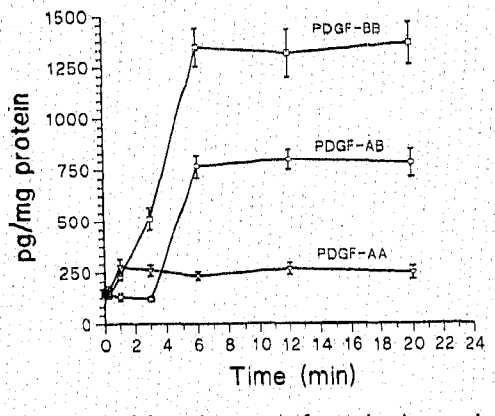

Fig. 4. Time course of thromboxane A_2 formation in vascular smooth muscle cells. Stimulation with 10 ng/ml PDGF-BB (upper trace), 10 ng/ml PDGF-BB (middle trace), and 10 ng/ml PDGF-AA (lower trace). Data from one representative experiment from three triplicate determinations are summarized (mean ± SE). The reaction was terminated by quickly freezing of the cell layer. Thromboxane A_2 was purified and determined according to the instructions of the thromboxane B_2 RIA-kit (New England Nuclear).

various time intervals with 10 ng/ml PDGF-isoforms. Maximal TXA_2 formation of 1.35, 0.67 and 0.25 ng/mg protein induced by PDGF-BB, PDGF-AB and PDGF-AA, respectively, were observed within 6 min (basal value 0.15 ng/mg protein). PDGF-BB (1.35 ng/mg protein) had a greater efficacy than PDGF-AB (0.67 ng/mg protein). PDGF-AA had a negligible effect on TXA_2.

4. DISCUSSION

Our results document that all three PDGS-isoforms induced contraction of aortic rings. In this context, PDGF-BB indicated a greater efficacy than PDGF-AB and PDGF-AA, whereas PDGF-AB showed a greater efficacy than PDGF-AA. Furthermore, PDGF-BB indicated a greater efficacy on $[Ca^{2+}]_i$, pH_i and TXA_2 than PDGF-AB, whereas PDGF-AA had no effects on $[Ca^{2+}]_i$ and pH_i. PDGF-AA had a negligible effect on TXA_2 formation.

Elevation of $[Ca^{2+}]_i$ is the main trigger for contraction of VSMC [6]. Recently, it was shown that a vasoactive agonist-induced biphasic change of the resting pH_i in VSMC also plays a role for contraction of VSMC. It was assumed that the initial acidification occurs due to vasoactive agonists-induced elevation of $[Ca^{2+}]_i$, whereas the secondary alkalinisation was linked to the activation of the Na^+/H^+ exchanger [13]. However, the Na^+/H^+ exchanger plays a role in a variety of cellular functions, including vascular contractility [14]. Another potent vasoconstrictor for VSMC is TXA_2, a metabolite of prostaglandin endoperoxides [8,9]. PDGF-BB showed a greater efficacy on $[Ca^{2+}]_i$, pH_i and TXA_2 formation than PDGF-AB. These results are in good agreement with our results, indicating a greater efficacy of PDGF-BB than PDGF-AB on contraction of aortic rings. On the other hand PDGF-AA induced contraction without increasing of $[Ca^{2+}]_i$ and pH_i. In the current work, PDGF-AA induced a negligible TXA_2 formation, which hardly explains the PDGF-AA-induced contraction. The PDGF-AA-induced contraction may be explained by the following observations.

Recently, we have shown that PDGF-AA was more potent than PDGF-BB in stimulating protein kinase C (PKC) [15]. As described by Nishizuka [16], the activation of different subspecies of PKC, leading to cellular responses, is thought to be biochemically dependent on Ca^{2+}, but under some conditions physiologically independent of a net increase of $[Ca^{2+}]_i$. In addition, recent reports show that a direct activation of PKC by tumor-promoting phorbol esters can induce contraction of VSMC without involving an elevation of $[Ca^{2+}]_i$. There are different mechanisms described by which PKC induces contraction of VSMC. Jiang and Morgan reported that resting $[Ca^{2+}]_i$ is sufficient and required to support phorbol ester-induced contraction in VSMC [17], whereas Itoh et al. conclude that 12-*o*-tetradecanoylphorbol-13-acetate (TPA), a potent phorbol ester, enhances the Ca^{2+} sensitivity of the contractile proteins [18]. Since PDGF-AA is more potent than PDGF-BB in stimulating protein kinase C (PKC), we can conclude that the PDGF-AA-induced contraction of VSMC, however, occurs via stimulation of PKC, without preceding elevation of $[Ca^{2+}]_i$ and biphasic change of pH_i.

In conclusion, PDGF-BB and PDGF-AB are similar and both have features characteristic of conventional vasoconstrictor-agonists that directly increase $[Ca^{2+}]_i$, activate that Na^+/H^+ exchanger and stimulate the TXA_2 formation, wheras PDGF-AA probably acts via PKC stimulation in VSMC.

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