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PDGF-BB Induced Modulation of Ca^{2+} -Activated K^+ Channels Involves a G-Protein Dependent Mechanism in Cultured Human Endothelial Cells

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Abstract: The present study investigated whether Platelet Derived Growth Factor (PDGF) modulates Ca^{2+} -activated K^+ channels with large conductance and examined possible signaling mechanisms. BK_{Ca} activity was analyzed using the patch-clamp technique in cultured human endothelial cells derived from umbilical cord veins (HUVEC). Continuous perfusion of HUVEC with 30 ng mL^{-1} PDGF caused a significant increase of BK_{Ca} open-state probability ($n=17$; $p<0.05$) after 3 min. This BK_{Ca} activation was inhibited by preincubating the cells with pertussis toxin (100 ng mL^{-1}). Despite the fact that the BK_{Ca} was activated by a release of Ca^{2+} from internal stores, this study could not observe a reduction of the open-state probability, if the internal release of Ca^{2+} was blocked using procaine ($n=13$; $p=\text{n.s.}$). In conclusion PDGF activates BK_{Ca} involving a G-protein dependent mechanism. This BK_{Ca} activation may play an important role in PDGF mediated effects on human endothelial cells.

Key words: Endothelial cell, Ca^{2+} -activated K^+ channel, G protein, procaine

INTRODUCTION

In health, vascular endothelium forms a multifunctional interface between circulating blood and the various tissues and organs of the body. It constitutes a selectively permeable barrier for macromolecules, as well as a nonthrombogenic surface that actively maintains the fluidity of blood. Furthermore it is a secretory active tissue, serving as the source of multiple factors^[1]. As an example the endothelium plays a crucial role in the regulation of vascular tone. In response to humoral factors or hemodynamic forces endothelial cells release vasodilating factors, including Nitric Oxid (NO), prostacyclin, as well as an Endothelium-derived Hyperpolarizing Factor (EDHF)^[2-4]. In the control of endothelial function itself, activation of Ca^{2+} -activated K^+ channels (K_{Ca}) induces endothelial hyperpolarization, which regulates the electrochemical driving force for Ca^{2+} that is necessary for the modulation of endothelial cell functions^[5]. This is further strengthened by the observation that inhibition of K_{Ca} and especially the large-conductance K_{Ca} (BK_{Ca}) completely blocks flow-induced vasodilation, endothelial proliferation and

synthesis of reactive oxygen species^[6-9]. Nevertheless, it is not clearly understood how activation of endothelial BK_{Ca} contributes to the control of vascular functions as BK_{Ca} is strongly active only upon depolarization and requires a substantial increase in intracellular Ca^{2+} concentration $[\text{Ca}^{2+}]_i$, especially at negative resting membrane potentials. Thus, it is likely that other intracellular second messengers in addition to $[\text{Ca}^{2+}]_i$ costimulate endothelial BK_{Ca} activity. Several intracellular second messengers besides Ca^{2+} have been shown to regulate the BK_{Ca} in endothelial- and Vascular Smooth Muscle Cells (VSMC)^[10-12]. For instance, Pertussis Toxin (PTX) sensitive G-proteins have been demonstrated to transduce BK_{Ca} -activation in endothelial cells, cyclic guanosine monophosphate (cGMP) stimulates channel activity presumably via BK_{Ca} phosphorylation through cGMP-dependent protein kinases^[11]. Moreover, nitric oxide independently of nitric oxide-mediated cGMP generation has been reported to directly activate a BK_{Ca} in VSMC^[13]. Platelet-derived Growth Factor (PDGF) is a dimeric molecule that exists as homodimers or heterodimers of related polypeptide chains (A and B). PDGF has been shown to play an important role in

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angiogenesis *in vivo* and vascular remodeling by influencing endothelial cell proliferation and function *in vitro*^[14,15].

Aim of the present study was to investigate whether PDGF modulates BK_{Ca} in human endothelial cells and to assess a possible contribution of Intracellular Ca²⁺-release and PTX-sensitive G-proteins in this context.

MATERIALS AND METHODS

Chemicals: Platelet Derived Growth Factor BB (PDGF), Iberiotoxin (IBX), Pertussis Toxin (PTX), procaine (all Sigma, Deisenhofen, Germany); EBM and cell culture supplements (Promo Cell, Heidelberg, Germany); FCS (PAA, Linz, Austria).

Isolation and culture of HUVEC: Endothelial cells were isolated from human umbilical cord veins as described previously^[16]. Cells were grown in Endothelial Cell Basal Medium (EBM) with the addition of 10% Fetal Calf Serum (FCS). The following supplements were added to the culture medium: 0.4% endothelial growth supplement heparin, epidermal growth factor 0.1 ng mL⁻¹, hydrocortison 1 µg mL⁻¹, basic fibroblast factor 1 ng mL⁻¹, gentamicin 50 µg mL⁻¹. All experiments were carried out using endothelial cells from subcultures four to eight.

Electrophysiological recordings: Single channel membrane currents were measured by means of the patch-clamp technique in cell-attached patches^[17]. Patch pipettes of borosilicate glass (Hilgenberg, Malsfeld, Germany) with a final resistance of 5-8 MΩ, when filled with pipette solution were used. For the recording a L/M-PC patch-clamp amplifier (List, Darmstadt, Germany) was used. Data were low-pass filtered at 1 kHz (6-pole Bessel filter) and digitalized (sample rate: 10 kHz) using a Digidata 1200A (Axon Instruments, Foster City, CA, USA) A/D converter and captured on the hard disk of an IBM-compatible personal computer. Analysis of the unitary currents was performed with pClamp 6.0.3. software (Axon Instruments, Foster City, CA, USA). Open-state probability (NPo) was calculated from the ratio between the channel open time and the total recording time. In case of PDGF (30 ng mL⁻¹) application intermittent recordings of BK_{Ca} were made up to 6 min. For all experiments HUVEC were maintained in an extracellular (bath) solution containing (mmol L⁻¹): NaCl 140; KCl 5; MgCl₂ 0.5; d-glucose 5.5; HEPES 10; CaCl₂ 1.5; pH 7.3 (with NaOH). In some experiments the following substances were added to the bath solution: 100 nmol L⁻¹ IBX, 30 ng mL⁻¹ PDGF. The standard pipette solution contained (mmol L⁻¹): K⁺-aspartate 110; KCl 30; HEPES 5;

MgCl₂ 1; pH=7.3 (with KOH). All experiments were conducted at room temperature (20-2°C).

Statistical analysis: Statistical significance for repeated measurements of NPo after PDGF application was determined by using a Friedman test (p<0.01; SPSS for Windows; release 10.0) followed by a Wilcoxon-test. Results are expressed as mean values±SEM.

RESULTS

Effect of PDGF BB on BK_{Ca}: In order to test whether external PDGF can modulate BK_{Ca}, recordings in cell-attached patches were carried out in which HUVEC were continuously perfused with a 30 ng mL⁻¹ PDGF containing bath solution, after a control recording in a PDGF free bath solution. Since BK_{Ca} activity was very low at low depolarizing test potentials, we only studied the channel behavior at holding potentials of +60 and +80 mV. The results of representative recording of BK_{Ca} activity after PDGF perfusion (Fig. 1A) revealed a significant

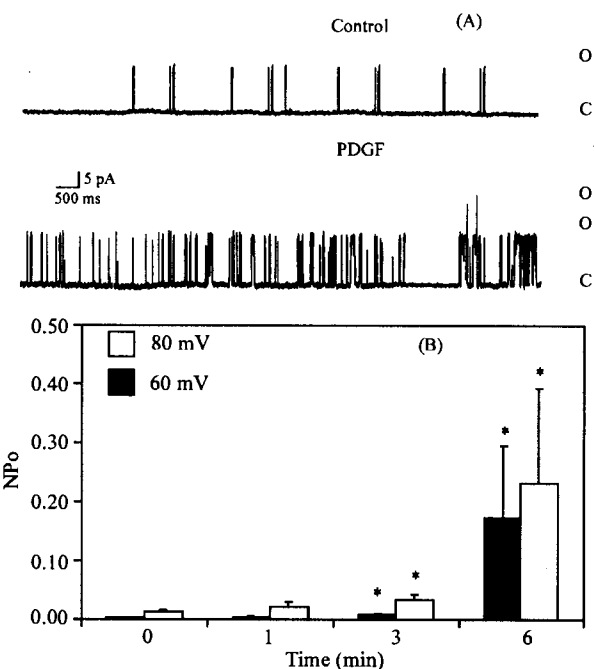


Fig. 1: PDGF increases BK_{Ca} activity in HUVEC

(A) Original recordings of BK_{Ca} in cell-attached patches after 3 min of perfusion with PDGF (30 ng mL⁻¹) at a pipette potential of +80 mV. The closed ● and open (○) states of the channel are indicated

(B) Plot of the open-state probability (NPo) of BK_{Ca} as a function of time prior to (0 min/control) and during continuous application of 30 ng mL⁻¹ PDGF at pipette potentials of +60 and +80 mV as indicated by vertical bars (n=17; *p<0.05 vs. control)

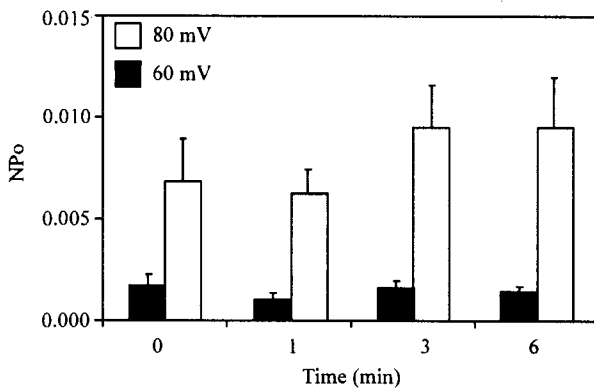


Fig. 2: Inhibition of PDGF-induced BK_{Ca} -activation by PTX

Plot of the open-state probability (NPo) of BK_{Ca} as a function of time prior to (0 min/control) and during continuous application of 30 ng/ml PDGF to PTX (100 ng mL^{-1}) pretreated HUVEC at pipette potentials of +60 and +80 mV as indicated by vertical bars ($n=14$; * $p<0.05$ vs. control)

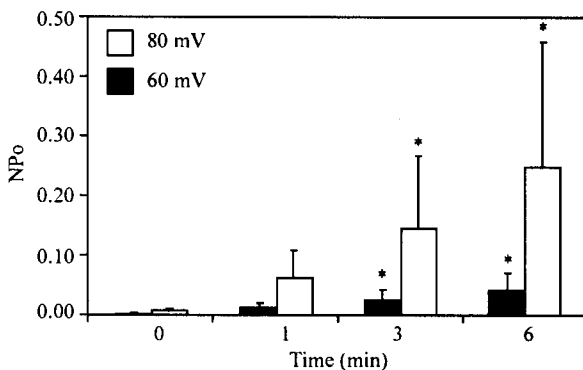


Fig. 3: The effect of PDGF on BK_{Ca} does not depend on a Ca^{2+} release from internal stores

Plot of the open-state probability (NPo) of BK_{Ca} as a function of time prior to (0 min/control) and during continuous application of 30 ng mL^{-1} PDGF to procaine (10 $\mu mol L^{-1}$) pretreated HUVEC at pipette potentials of +60 and +80 mV as indicated by vertical bars ($n=13$; * $p<0.05$ vs. control)

effect of PDGF on open-state probability (NPo) concerning the recording time. The paired comparisons for the various recording times during PDGF application versus control (without PDGF) resulted after 3 min in a significant increase of BK_{Ca} activity. Using a test potential of +60 mV the open-state probability was significantly increased from 0.001 ± 0.001 at control conditions to 0.004 ± 0.001 ($n=17$; $p<0.05$) after 3 min of

PDGF BB treatment. When applying test potentials of +80 mV, NPo was significantly increased from 0.009 ± 0.002 (control) to 0.030 ± 0.010 ($n=17$; $p<0.05$) after 3 min of PDGF perfusion (Fig. 1B). Single-channel slope conductance was not affected by PDGF (168.6 ± 3.1 pS vs. 166.8 ± 4.1 pS; $n=4$; n.s.).

To verify that the highly selective BK_{Ca} blocker IBX is sufficient to block PDGF induced BK_{Ca} activation we added 100 $nmol L^{-1}$ IBX to the pipette solution and carried out cell-attached measurements of BK_{Ca} . Within 2 min after gigaseal formation we observed a total block of initial BK_{Ca} openings which assures the existence of BK_{Ca} in the patch at holding potentials of +80 mV. The following application of PDGF BB did not alter the blockade of BK_{Ca} by IBX ($n=9$, data not shown).

PDGF induced BK_{Ca} activation involves G-proteins: To examine whether PDGF-mediated activation of endothelial BK_{Ca} involves G-proteins this study performed cell-attached measurements after preincubation of HUVEC with the G-protein inhibitor pertussis toxin (PTX; 100 ng mL^{-1}). Under this condition PDGF (30 ng mL^{-1}) superfusion did not cause a significant increase in BK_{Ca} activity at test potentials of +60 mV and +80 mV (Fig. 2).

Procaine does not block PDGF induced BK_{Ca} activation: Procaine has been shown to inhibit Ca^{2+} release from internal stores in endothelial cells^[18]. Since increases of intracellular Ca^{2+} can contribute to BK_{Ca} activation, we tested whether preincubation of HUVEC with procaine (Pro; 10 $mmol L^{-1}$) inhibits PDGF induced ion channel activation. After 6 min of continuous PDGF perfusion BK_{Ca} open-state probability was significantly increased in procaine treated cells. At a test potential of +60 mV NPo was increased from 0.001 ± 0.001 (control) to 0.043 ± 0.028 (PDGF+Pro) and at a test potential of +80 mV from 0.006 ± 0.003 (control) to 0.248 ± 0.216 (PDGF+Pro) (Fig. 3).

DISCUSSION

This study described the mechanism of activation of large conductance Ca^{2+} -activated K^{+} channels in cultured HUVEC by PDGF. Electrophysiological data shows for the first time that the angiogenic growth factor PDGF strongly activates BK_{Ca} in human endothelial cells involving a PTX-sensitive signaling pathway.

Electrophysiological experiments to identify Ca^{2+} -activated K^{+} channels in HUVEC are in line with the description of BK_{Ca} in other studies^[12,19,20]. Using cell-attached patches, we observed a significant activation of BK_{Ca} in human endothelial cells by PDGF.

Continuous PDGF perfusion caused a significant increase of BK_{Ca} activity after 3 min at test potentials of +60 mV and +80 mV, respectively. To obtain information about the mechanisms of endothelial BK_{Ca} activation after binding of PDGF to its specific receptor, we tested a possible contribution of G-proteins in the signaling pathway. Results clearly demonstrated that PTX-sensitive G-proteins are involved in PDGF-induced modulation of endothelial BK_{Ca}. These results are consistent with recent findings of Begg *et al.*^[12]. They showed that atypical cannabinoids induce BK_{Ca} activation via PTX-sensitive G proteins in cultured HUVEC. Nevertheless, activation of BK_{Ca} by PTX-sensitive G-proteins is not the only possible mechanism that might be responsible for the effect of PDGF. It has been shown that VEGF induced activation of BK_{Ca} in HUVEC depends on an increase of intracellular Ca²⁺ concentrations, which was mediated by a release of Ca²⁺ from the endoplasmatic reticulum^[19]. Therefore, the hypothesis was tested that PDGF induced BK_{Ca} activation depends on Ca²⁺ release from internal stores. For this purpose procaine was used, which has been shown to block Ca²⁺ release from the endoplasmatic reticulum^[18]. In present experiments treatment of HUVEC with procaine failed to inhibit PDGF induced ion channel activation. For this reason the conclusion can be drawn that PDGF mediated modulation of endothelial BK_{Ca} is more likely caused by G proteins, then by a Ca²⁺ release from internal stores.

An activation of a nonselective cation channel in fibroblasts has been reported, which was of functional relevance, since a block of this ion channel inhibited PDGF-induced fibroblast proliferation^[21]. Up to now, the complete electrophysiological properties of PDGF on endothelial cells remain unclear. Under physiological conditions, activation of endothelial BK_{Ca} increases the K⁺ permeability, hyperpolarizing the endothelial cell and thereby increases the electrochemical gradient for maintained Ca²⁺ entry during agonist stimulation^[5,22]. Therefore this study concluded, that the modulation of BK_{Ca} by PDGF might have functional relevance, as it was demonstrated in other studies. As an example it was demonstrated that BK_{Ca} activation contributes to cerivastatin-induced release of nitric oxide^[23], furthermore oxidized low density lipoprotein-and lysophosphatidylcholine-dependent proliferation of HUVEC was reduced, if the BK_{Ca} inhibitor IBX was applied^[8,9].

In conclusion the results of the present patch-clamp study demonstrated that PDGF activates BK_{Ca} in cultured HUVEC involving a G protein coupled signaling cascade. In future studies this mechanism might be of interest for the investigation of other PDGF-induced endothelial signaling pathways.

REFERENCES

1. Kuruvilla, L. and C.C. Kartha, 2003. Molecular mechanisms in endothelial regulation of cardiac function. *Mol. Cell. Biochem.*, 253: 113-123.
2. Palmer, R.M.J., A.G. Ferrige and S. Moncada, 1987. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*, 327: 524-526.
3. Rubanyi, G.M., J.C. Romero and P.M. Vanhoutte, 1986. Flow-induced release of endothelium-derived relaxing factor. *Am. J. Physiol.*, 250: 1145-1149.
4. Mombouli, J.V. and P.M. Vanhoutte, 1997. Endothelium-derived hyperpolarizing factor(s): Updating the unknown. *Trends Pharmacol. Sci.*, 18: 252-256.
5. Nilius, B. and G. Droogmans, 2001. Ion channels and their functional role in vascular endothelium. *Phys. Rev.*, 81: 1415-1459.
6. Cooke, J.P., E. Rossitch, N. Andon, J. Loscalzo and V.J. Dzau, 1991. Flow activates an endothelial potassium channel to release an endogenous nitrovasodilator. *J. Clin. Invest.*, 88: 1663-1671.
7. Hutcheson, I.R. and T.M. Griffith, 1994. Heterogenous populations of K⁺ channels mediate EDRF release to flow but not agonist in rabbit aorta. *Am. J. Physiol.*, 266: 590-599.
8. Kuhlmann, C.R., M. Schäfer, F. Li, T. Sawamura, H. Tillmanns, B. Waldecker and J. Wiecha, 2003. Modulation of endothelial Ca²⁺-activated K⁺ channels by oxidized LDL and its contribution to endothelial proliferation. *Cardiovasc. Res.*, 60: 626-634.
9. Kuhlmann, C.R., D.W. Lüdders, C.A. Schaefer, A.K. Most, U. Backenköhler, T. Neumann, H. Tillmanns and A. Erdogan, 2004. Lysophosphatidylcholine induced modulation of Ca²⁺-activated K⁺ channels contributes to ROS dependent proliferation of cultured human endothelial cells. *J. Mol. Cell. Cardiol.*, 36: 675-682.
10. Schubert, R. and M.T. Nelson, 2001. Protein kinases: Tuners of the BK_{Ca} channel in smooth muscle. *Trends Pharmacol. Sci.*, 22: 505-512.
11. Fukao, M., H.S. Mason, F.C. Britton, J.L. Kenyon, B. Horowitz and K.D. Keef, 1999. Cyclic GMP-dependent protein kinase activates cloned BK_{Ca} channels expressed in mammalian cells by direct phosphorylation at serine 1072. *J. Biol. Chem.*, 274: 10927-10935.
12. Begg, M., F.M. Mo, L. Offertaler, S. Batkai, P. Pacher, R.K. Razdan, D.M. Lovinger and G. Kunos, 2003. G protein-coupled endothelial receptor for atypical cannabinoid ligands modulates a Ca²⁺-dependent K⁺ current. *J. Biol. Chem.*, 278: 46188-46194.

13. Bolotina, V.M., S. Najibi, J.J. Palacino, P.J. Pagano and R.A. Cohen, 1994. Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle. *Nature*, 368: 350-353.
14. Battergay, E.J., J. Rupp, L. Iruela-Arispe, E.H. Sage and M. Pech, 1994. PDGF-BB modulates endothelial proliferation and angiogenesis *in vitro* via PDGF beta-receptors. *J. Cell. Biol.*, 125: 917-928.
15. Orlandi, C., A.J. Paskins-Hurlburt and N.K. Hollenberg, 1986. The microvascular response to growth factors in the hamster cheek pouch. *Basic. Res. Cardiol.*, 81: 238-243.
16. Jaffe, E.A., R.L. Nachmann, C.G. Becker and C.R. Minick, 1973. Culture of human endothelial cells derived from umbilical veins-identification by morphological and immunological criteria. *J. Clin. Invest.*, 52: 2745-2756.
17. Hamill, O., A. Marty, E. Neher, B. Sakmann and F. Sigworth, 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers. Arch.*, 391: 85-100.
18. Thuringer, D. and R. Sauve, 1992. A patch-clamp study of the Ca^{2+} mobilization from internal stores in bovine aortic endothelial cells. I. Effects of caffeine on intracellular Ca^{2+} stores. *J. Membr. Biol.*, 130: 125-137.
19. Faehling, M., E.D. Koch, G. Raithel, G. Trischler and J. Waltenberger, 2001. Vascular endothelial growth factor-A activates Ca^{2+} -activated K^{+} channels in human endothelial cells in cultur. *Intl. J. Biochem. Cell. Biol.*, 33: 337-346.
20. Sauve, R., L. Parent, C. Simoneau and G. Roy, 1988. External ATP triggers a biphasic activation of a calcium-dependent K^{+} channel in cultured aortic bovine endothelial cells. *Pflugers. Arch.*, 412: 469-481.
21. Jung, F., S. Selvaraj and J.J. Gargus, 1992. Blockers of platelet-derived growth factor-activated nonselective cation channel inhibit cell proliferation. *Am. J. Physiol.*, 262: 1464-1470.
22. Adams, D.J., 1994. Ionic channels in vascular endothelial cells. *Trends. Cardiovasc. Med.*, 4: 18-26.
23. Kuhlmann, C.R., C. Gast, F. Li, M. Schäfer, H. Tillmanns, B. Waldecker and J. Wiecha, 2004. Cerivastatin activates endothelial calcium-activated potassium channels and thereby modulates endothelial NO production and cell proliferation. *J. Am. Soc. Nephrol.*, 15: 868-875.