Vascular Dopamine-I Receptors and Atherosclerosis

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Vascular smooth muscle cell (VSMC) migration and proliferation are believed to play key roles in atherosclerosis. To elucidate the role of vascular dopamine D1-like receptors in atherosclerosis, the effects of dopamine, specific D1-like agonists SKF 38393, and YM 435 on platelet-derived growth factor (PDGF) BB mediated VSMC migration, proliferation, and hypertrophy were studied. We observed that cells stimulated by 5 ng/ml PDGF BB showed increased migration, proliferation and hypertrophy. These effects were prevented by coincubation with dopamine, SKF 38393, or YM 435 at 1-10 μmol/l, and this prevention was reversed by Sch 23390 (1-10 μmol/l), a specific D1-like antagonist. These actions are mimicked by 1-10 μmol/l forskolin, a direct activator of adenylate cyclase and 8-bromo-cyclic AMP at 0.1-1 mmol/l. The actions are blocked by a specific protein kinase A (PKA) inhibitor N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinoline-sulfonamide (H 89), but are not blocked by its negative control, N-[2-(N-formyl-p-chlorocinnamylamino)ethyl]-5-isoquinoline sulfonamide (H 85). PDGF-BB (5 ng/ml) mediated activation of phospholipase D (PLD), protein kinase C (PKC) and mitogen-activated protein kinase (MAPK) activity were significantly suppressed by coincubation with dopamine. These results suggest that vascular D1-like receptor agonists inhibit migration, proliferation and hypertrophy of VSMC, possibly through PKA activation and suppression of activated PLD, PKC and MAPK activity. J Atheroscler Thromb, 1997; 4: 59-64.

Key words: Dopamine, Vascular smooth muscle, Migration, Proliferation

Introduction

In 1972, Goldberg et al. reported on the cardiovascular and renal actions of dopamine, and predicted the existence of dopamine-I receptors on renal, coronary and mesenteric vascular beds (1). Later, we reported on the existence of dopamine-I receptors on renal vascular smooth muscle cells using biochemical methods (2-4).

Two distinct classes of dopamine receptor were originally thought to exist in peripheral tissue, and are designated as D1- and D2-like receptors (5). At least 5 dopamine receptor subtypes are cloned from the brain. Types D1A and D1B are D1-like, whereas types D2, D3 and D4 are D2-like (6-8). Further, D1A receptors have been reported in coronary arterial smooth muscle cells (9), and D1-like receptors have been biochemically evaluated (10, 11). Stimulation of D1-like receptors causes vasodilation (12), which is consistent with the evidence that the blood pressure of dopamine-I receptor defect mice is higher than that of control mice (13). Vasodilator hormones such as natriuretic peptides and β-adrenergic receptor agonists have been shown to act as the anti-growth factors (14, 15).

This paper reviews the possible role of the D1-like receptors on PDGF-BB-mediated VSMC migration and proliferation, and examines the potential therapeutic effect of D1-like receptor agonists on atherosclerosis.
Existence of dopamine-I receptors on VSMC and their modulation by various vasoactive substances

Ozono et al. have demonstrated that the gene and protein of the D1A receptor are expressed in the coronary arteries (9). The modulation of dopamine D1-like receptors of cultured rat renal VSMC by phorbol ester, glucocorticoid and sodium chloride was studied. The extent of [3H] Sch-23390 binding to phorbol ester-treated VSMC was increased without any change in the dissociation constant (Kd). At a concentration of 10 nmo1/l, the synthetic glucocorticoid dexamethasone increased maximum receptor binding (Bmax) but had no effect on the Kd. 100 mmol/l sodium chloride did not change Bmax, but increased the Kd for the D1-like receptor. The production of cyclic AMP (cAMP) in response to D1-like receptor stimulation was enhanced without any change in the adenylate cyclase activity. The glucocorticoid effect on the D1-like receptor in VSMC became apparent after several hours of incubation in the presence of the steroid and was significantly inhibited by cycloheximide (10 ,ug/ml) and by the glucocorticoid receptor antagonist RU-38486, indicating that the effect required protein synthesis through glucocorticoid receptors. Treatment of VSMC with 1 ,umol/l dexamethasone for 24 h increased basal and D1-like-stimulated adenylate cyclase activity. Basal adenylate cyclase was decreased by sodium chloride in a dose-dependent manner. These results suggest the evidence of differential control of D1-like receptors in VSMC by protein kinase C, glucocorticoid, or sodium chloride (2, 3, 16, Table).

Antimigration of VSMC by dopamine-I receptor activation

The migration of arterial medial VSMC into the intima is important in intimal thickening, not only in atherosclerotic lesions but also in restenosis after angioplasty (17). The effect of the D1-like receptor agonists, dopamine, SKF 38393, and YM 435, on the migration of VSMC treated with PDGF-BB for 4 h was studied (18). D1-like receptor agonists clearly inhibited PDGF-BB (5 ng/ml)-induced VSMC migration in a concentration-dependent manner. Although non-stimulated VSMC exhibited little migration activity, the D1-like receptor agonists did not inhibit this basal activity. There was a significant correlation between percentage increase in D1-like agonists-mediated cAMP formation and the percentage decrease in migration activity (r = 0.89, p < 0.01).

This study has demonstrated for the first time that D1-like agonists inhibit the migration and proliferation of VSMC stimulated with PDGF in a concentration-dependent manner. PDGF-BB-induced VSMC migration and proliferation were inhibited significantly by 1-10 ,umol/l dopamine. Inhibition percentages by 10 ,umol/l dopamine, 10 ,umol/l SKF 38393, and 10 ,umol/l YM 435 of VSMC migration stimulated with 5 ng/ml PDGF-BB were 31%, 20% and 43%, respectively.

It has been reported that stimulation of phosphatidylinositol turnover, diacylglycerol formation, and intracellular Ca2+ flux are likely to be required for the chemotaxis of human VSMC (19). Since PKA activation decreases PLD, and cAMP decreases the intracellular Ca2+ level in VSMC (20), it is possible that the decreased diacylglycerol formation resulting from suppression of PLD by PKA activation, or the decreased cytosolic Ca2+ induced by cAMP suppresses the migratory activities of VSMC stimulated with PDGF-BB. However, the exact cellular mechanism by which cAMP inhibits PDGF-BB-induced migration of VSMC has yet to be fully understood. Very recently, it was reported that MAPK activation is involved in PDGF-directed migration in VSMC (21). Therefore, inhibition of PDGF-directed migration by D1-like agonists may be explained by PKA-mediated inhibition of MAPK activity (Figure).

Antiproliferative action of vascular dopamine-I receptors

Proliferation of VSMC is thought to play a key role in the development of atherosclerotic lesions (17). Understanding the regulatory mechanism of VSMC proliferation is, therefore, important in elucidating the pathogenesis of atherosclerosis, and also in the design of therapeutic drugs or devices for treating therogenous disorders.
The number of cells stimulated by PDGF-BB over 48 h was higher than that stimulated by a control (vehicle). The D1-like agonists; dopamine, SKF 38393 and YM 435, was higher than that stimulated by a control (vehicle). Moreover, we have recently demonstrated that dopamine inhibits PDGF-BB-induced DNA synthesis in VSMC has been also reported (37). It is possible that dopamine inhibits VSMC migration and proliferation stimulated by PDGF-BB, probably through a cAMP-dependent process and PKA activation (18).

Cyclic AMP inhibits serum- or PDGF-BB-induced DNA synthesis in VSMC, therefore affecting cell proliferation (22, 23). Moreover, we have recently demonstrated that adrenomedulin, which increases cAMP in VSMC, inhibits the FCS-induced proliferation of VSMC (24) and it is possible that cAMP elevation by D1-like agonists also inhibits PDGF-induced proliferation of VSMC. We have demonstrated for the first time that dopamine-induced PKA activation inhibit activation of PLD, PKC, and MAPK by PDGF (18). Previous reports that PKA mediates inhibition of PDGF-BB-induced by MAPK signaling in VSMC (25) are consistent with the present study. The antagonism by PKA does not appear to be at the level of the PDGF receptor β subunit, MAPK kinase, or MAPK, but is likely to occur between the receptor and MAPK kinase. A number of upstream activators of MAPK kinase have been reported, including Raf-1 (26). It has been reported that activation of PKC is required to activate MAPK (27–29) as shown in the study (18), and to activate hyperphosphorylation of Raf-1 in VSMC (30), and that activation of Raf-1 is inhibited by PKA in Raf-1 fibroblast cells (31, 32). PKC may therefore contribute to the suppression of the MAPK activity by PKA. Since PLD activates PKC through the formation of diacylglycerol in VSMC (33), PLD also may contribute to this suppression. However, since PLD is also reported to be activated by PKC (34), the possibility that PLD suppression is the result of PKC suppression cannot be excluded. Possible sites of action remain to be clarified. In rat glioma C6 Bul cells, phospholipase C-γ1, which plays an important role in mediating the PDGF mitogenic signal (35), is reported to be phosphorylated by PKA (36). Further, the D1-like receptor agonist fenoldopam is reported to decrease PLC-γ1 activity in renal tissue (37), and inhibition by cAMP of basal and induced inositol phosphate production in VSMC has been also reported (37). It is possible that activation of D1-like agonist-mediated PKA phosphorylates PLC-γ1 and affects PLD and PKC signal transduction, which, in turn, suppress the MAPK pathway. Moreover, as shown in Table 2, PDGF-BB significantly changed the cell cycle from G0-G1 to the S phase. Activation of MAPK isoforms is required for cell cycle progression and S phase entry of fibroblast in response to mitogenic factors (39). The D1-like agonists inhibited this progression. These results indicate that D1-like agonists activate PKA, which modifies PLD, PKC and MAPK activity. These changes, in turn, suppress the cell cycle and cell proliferation.

Dopamine-I receptors and hypertrophy of vascular smooth muscle cells

Excessive VSMC growth has been highlighted recently in the pathophysiology of hypertension and atherosclerosis. Indeed, one of the hallmarks of chronic hypertension is a generalized increase in the smooth muscle mass of the blood vessel wall (40). Whereas acute hypertensive models (such as aortic coarctation) or experimental injury models of atherosclerosis are characterized by VSMC proliferation (hyperplasia) (41, 42), chronic hypertension models such as the Goldblatt two-kidney, one-clip hypertensive rats and spontaneously hypertensive rats exhibit aortic VSMC hypertrophy, showing an increase in polyploidy without an increase in cell number (43, 44). PDGF is one of the major mitogens in serum and is responsible for the proliferation of certain cell types, including VSMC (45). It has been reported that the

Figure Regulation of dopamine-I receptor-mediated inhibition of VSMC migration, proliferation, and hypertrophy. Dopamine D-I like receptors activate adenylate cyclase and protein kinase A, which suppresses phospholipase D (PLD), protein kinase C (PKC) and mitogen activated protein kinase (MAPK) activity, which in turn may result in the suppression of VSMC migration, proliferation and hypertrophy.
PDGF-BB isoform is a potent inducer of hypertrophy, as well as hyperplasia, of VSMC. Therefore, the study was designed to investigate the possible role of the D1-like receptors on PDGF-BB-mediated VSMC hypertrophy, and to examine the potential therapeutic significance of D1-like receptor agonists on atherosclerosis.

This study has demonstrated for the first time that D1-like receptor agonists inhibit the hypertrophy of VSMC stimulated with PDGF in a concentration-dependent manner (46). PDGF-BB-induced VSMC hypertrophy was significantly inhibited by 1-10 \( \mu \text{mol/l} \) dopamine. Dopamine and YM 435 completely and SKF 38393 partially prevented PDGF-BB (5 ng/ml)-induced hypertrophy of VSMC, estimated by \( [\text{H}] \) leucine incorporation and cell size measured by flow cytometry (47, 48). Although it has been reported that immunoreactive dopamine is present in human and rat plasma (49, 50), the plasma free dopamine concentrations (\( \approx 0.1 \) to \( 1 \) nmol/l) are much lower than the levels of dopamine required to significantly inhibit VSMC hypertrophy in our in vitro study.

We have obtained some evidence for a causal link between an increase in cAMP production and the inhibition of VSMC hypertrophy treated with PDGF-BB. Forskolin, an activator of adenylate cyclase, and 8-bromo-cAMP, a cAMP analogue, prevented a PDGF-induced increase in \( [\text{H}] \) leucine incorporation and relative cell size. Moreover, PKA inhibitor H 89 reversed this D1-like receptor agonist-mediated inhibition of protein synthesis. These results suggest that dopamine inhibits VSMC hypertrophy stimulated by PDGF-BB, probably through a cAMP-dependent process and PKA activation.

It has been reported that not only proliferative agents but also hypertrophic agents such as thromboxane A (51), or angiotensin II (27) induce MAPK activation. PKA antagonization of PDGF-induced signaling by MAPK in human VSMC has also been identified (25). We have obtained results indicating that PKA activation by the stimulation of D1-like receptors reduced MAPK activity, and suggest that this inhibition may play some role in the antihypertrophic action of D1-like receptor agonists (Figure).

Activation of D1-like receptor suppresses PDGF-BB-mediated VSMC hypertrophy through PKA activation and inhibition of activated MAPK activity.

**Conclusion**

In conclusion, activation of D1-like receptors suppresses PDGF-BB-mediated VSMC migration, proliferation and hypertrophy by activating PKA and suppressing PLD, PKC and MAPK activity.

We have examined VSMC from the renal artery (18, 46). Since approximately two-thirds of all lesions of renovascular hypertension in adults are caused by atherosclerosis (52), this protective effect of the D1-like agonists may have a potential therapeutic significance for prevention of this disease. However, whether the D1-like agonists will prevent the development of atherosclerosis is another critical question raised by these studies. VSMC proliferation is an intermediate event in the process of atherogenesis, which follows endothelial cell damage and the deposition of fatty streaks in the vessel wall, and results in the formation of the organized atherosclerotic plaque (53). Further in vivo study will be necessary to elucidate the potential therapeutic significance of dopamine-I agonists.

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