

## Growth Factor-Induced Amino Acid Uptake by Vascular Smooth Muscle Cells

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### Abstract

Although accumulating evidence suggests that phosphatidylinositol 3-kinase (PI3K) is a common signaling molecule for growth factor-induced amino acid uptake by the cell, the role of PI3K in the uptake of different amino acids was not tested under the same conditions. In this study, we asked whether PI3K mediates platelet-derived growth factor (PDGF)-stimulated uptake of different amino acids that are taken up through 3 major amino acid transporters expressed in rat vascular smooth muscle cells and other cell types and whether PI3K mediates amino acid uptake stimulated with different growth factors and vasoactive substances. PDGF increased the uptake of [3H] leucine, [3H] proline, and [3H] arginine in a dose- and time-dependent fashion. Two different PI3K inhibitors, wortmannin (100 nmol/L) and LY294002 (10  $\mu$ mol/L), completely inhibited the amino acid uptake stimulated by PDGF. Chinese hamster ovary cells expressing both PDGF receptor- $\beta$  and a dominant-negative PI3K did not increase their leucine uptake when stimulated with PDGF, whereas the same cells expressing only PDGF receptor- $\beta$  did. Transforming growth factor- $\beta$ , as well as insulin-like growth factor-I and angiotensin II, increased leucine uptake by vascular smooth muscle cells. Wortmannin and LY294002 inhibited this increase. We also found that transforming growth factor- $\beta$  stimulated PI3K activity and the phosphorylation of Akt, a downstream signaling molecule of PI3K. A similar effect of PI3K inhibitors on amino acid uptake was observed in Swiss 3T3 cells. We conclude that PI3K mediates the uptake of different amino acids by vascular smooth muscle cells and other cell types stimulated with a variety of growth factors, including transforming growth factor- $\beta$ . Our findings suggest that PI3K may play an important role in vascular pathophysiology by regulating amino acid uptake.

**Keywords:** Phosphatidylinositol 3-kinase; Amino acid uptake; Platelet-derived growth factor; Transforming growth factor- $\beta$ .

### Introduction

Involvement of phosphatidylinositol 3-kinase (PI3K) in amino acid uptake has been reported with different cell types and growth factors: insulin-stimulated uptake of  $\alpha$ -aminoisobutyric acid in VSMCs and skeletal muscle, uptake of  $\alpha$ -aminoisobutyric acid and leucine in mouse 3T3 fibroblasts; uptake of methyl  $\alpha$ -aminoisobutyric acid in 3T3-L1 adipocytes; and PDGF-induced uptake of leucine in Swiss 3T3 cells. These studies suggest that PI3K is a common signaling molecule in the uptake of various amino acids by various cell types. However, these studies were performed under different conditions with different cell types, and the notion was never tested in a single cell type. In this study, we asked whether PI3K is a common signaling molecule for the uptake of amino acids through different transporters expressed in VSMCs and Swiss 3T3 cells and whether PI3K is a common signaling molecule for different stimuli of amino acid uptake in these cells [1].

### PDGF Stimulates VSMC Uptake of 3 Different Amino Acids through PI3K

PDGF-BB significantly stimulated the uptake by VSMCs of leucine, arginine, and proline, which are mainly taken up by system L, CAT, and system A, respectively (Figure 1). The time course and dose dependency were similar to those reported previously by others.<sup>16</sup> The amino acid uptake by PDGF-stimulated VSMCs reached a plateau at 4 hours, with maximum values of a 1.4-fold increase over controls for leucine, 1.7-fold for arginine, and 1.8-fold for proline (Figure 1). The uptake of all amino acids was stimulated with 1 to 5 ng/mL PDGF-BB, and higher concentrations did not further increase the uptake. A similar increase was observed in Swiss 3T3 cells (data not shown).

Wortmannin (100 nmol/L) and LY294002 (10  $\mu$ mol/L), 2 inhibitors of PI3K with different modes of action, completely inhibited

uptake of the 3 amino acids in both VSMCs (Figure 2A) and Swiss 3T3 cells (Figure 2B) at concentrations sufficient to inhibit PI3K activity in these cells, indicating that PI3K mediates amino acid uptake through different amino acid transporting systems [2].

### CHO Cells Expressing PDGFR and a Dominant-Negative Subunit of PI3K

To rule out the possibility that the above findings were due to nonspecific effects of the inhibitors, we prepared CHO cells expressing PDGFR- $\beta$  and a dominant-negative p85 subunit of PI3K, and studied the effect of PI3K suppression on PDGF-induced leucine uptake. A stable cell line of CHO cells expressing PDGFR- $\beta$  alone (CHO/PDGFR) and CHO cells expressing both PDGFR- $\beta$  and a dominant-negative p85 subunit of PI3K (CHO/PDGFR/ $\Delta$ p85) had functional PDGFR- $\beta$ ; PDGF-BB tyrosine-phosphorylated PDGFR- $\beta$  and activated phospholipase C- $\gamma$ , a downstream signal, in these cells. The  $\Delta$ p85 subunit co-transfected with PDGFR- $\beta$  effectively inhibited the PDGF-induced activation of PI3K; the p110 subunit became associated with PDGFR in CHO/PDGFR cells, whereas it did not in CHO/PDGFR/ $\Delta$ p85 cells. PI3K activity measured with PI as the substrate was suppressed in CHO/PDGFR/ $\Delta$ p85 cells to <5% of that in CHO/PDGFR

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cells. PDGF-BB did not stimulate leucine uptake in CHO/PDGFR/ $\Delta$ p85 cells but stimulated it in CHO/PDGFR cells in a dose-dependent fashion, confirming the findings obtained with the inhibitors [3].

## Discussion

An important finding of the current study is that PI3K is a common signaling molecule that transmits the stimulus from the growth factor receptor to different amino acid transport systems. Different amino acids are taken up through different amino acid transporters that are regulated differently and have been implicated in different biological consequences. Leucine is mainly taken up by VSMCs through the system L amino acid transporter, which is 1 of the ubiquitous transporters regulated partly by nonhormonal mechanisms and that plays a role in general protein synthesis in VSMCs. Arginine is taken up by VSMCs through 2 subtypes of a CAT, CAT-1 and CAT-2B. This transporter activity is important in poly-amine synthesis required for PDGF-induced mitogenesis and in providing arginine to NO synthase located at the caveolae. In mammals, proline is taken up through system A, another ubiquitous transporter serving for most bipolar amino acids [4-7]. Previous reports from our laboratory and others have indicated that PI3K plays an important role in amino acid uptake through an individual transport system. However, the uptake of different amino acids was tested with different cell types but was never compared in any single cell type. Neither had a role for PI3K in amino acid transport through CAT been documented. The current study demonstrates that PI3K is indispensable for the stimulation of different amino acid transport systems, including CAT. Because previous studies had indicated that amino acid uptake is important for cellular proliferation, vascular hypertrophy, vascular remodeling, and regulation of vascular tone, PI3K may be a key enzyme in a wide range of VSMC functions [8].

Another important finding is that different growth factors and vasoactive substances share PI3K as a common signaling molecule in their stimulation of amino acid uptake. PDGF and IGF-I have receptors coupled with tyrosine kinases and transmit their signal through tyrosine phosphorylation of signaling molecules that have src homology domains in them. TGF- $\beta$  transmits its signal through non-tyrosine kinase-type receptors and through unique signaling molecules, such as SMAD and TAK1 (TGF- $\beta$ -activated kinase-1). Ang II transmits its signal through receptors coupled with G proteins and protein kinase C. Despite all of these differences in signal transduction, amino acid uptake with different stimuli was blocked by the PI3K inhibitors [9]. The role of PI3K in amino acid uptake is not limited to VSMCs but also occurs in other cell types, such as Swiss 3T3 and CHO cells (Figure 2).

These conclusions were based on experiments with both PI3K inhibitors and with CHO cells expressing a dominant-negative PI3K. Wortmannin, a noncompetitive and irreversible inhibitor of PI3K, and LY294002, a competitive inhibitor, have been used to inhibit PI3K activity in various cells and to study the physiological role of PI3K. Both compounds inhibit PI3K activity of the purified enzyme and in cultured cells at 100 nmol/L, a concentration insufficient to inhibit phospholipase A<sub>2</sub> or myosin light-chain kinase. To further rule out the possibility that the inhibition of amino acid uptake was due to nonspecific effects of the inhibitors, we prepared CHO cells expressing both PDGFR- $\beta$  and a dominant-negative PI3K. These cells expressed more PDGFR and responded more to PDGF than did the CHO/ $\Delta$ p85 cells that we had reported previously. The amount of PDGFR protein and the tyrosine phosphorylation of PDGFR and phospholipase C- $\gamma$  are comparable between CHO/PDGFR/ $\Delta$ p85 and CHO/PDGFR. PI3K activation and leucine uptake were completely suppressed in CHO/PDGFR/ $\Delta$ p85

cells, confirming the findings obtained with the inhibitors [10].

Although PDGFR and insulin receptor substrate-1 (IRS-1), a downstream signaling molecule of the IGF-I receptor, directly bind to PI3K and increase its activity, it is not clear how the receptors for Ang II and TGF- $\beta$  stimulate PI3K activity. Ang II increases tyrosine phosphorylation of IRS-1 and the subsequent association of IRS-1 and PI3K in the rat heart. However, Ang II does not increase PI3K activity in that model. Recently, it was revealed that Ang II phosphorylates and activates growth factor receptors, such as the receptor for epidermal growth factor. Ang II may activate PI3K indirectly by activating other growth factor receptors as well [11-13].

So far as we are aware, this is the first report showing that TGF- $\beta$  increases PI3K activity. TGF- $\beta$  increased PI3K activity in anti-PI3K immune precipitates by 20%. This rather small increase is similar to that obtained with PDGF stimulation and reflects a large pool of PI3K that is unaffected by a single growth factor. TGF- $\beta$  did not increase PI3K activity in the anti-phosphotyrosine immunoprecipitate, whereas PDGF increased it 20-fold, suggesting that PI3K activation with TGF- $\beta$  is not mediated by tyrosine phosphorylation. Because we could not immunoprecipitate PI3K with an anti-TGF- $\beta$  type II receptor, TGF- $\beta$  may stimulate PI3K indirectly. Activation of PI3K by TGF- $\beta$  was further substantiated by phosphorylation of a downstream signaling molecule of PI3K, Akt, which is phosphorylated at serine 473 by PI 3,4-bisphosphate, a product of activated PI3K [14].

In summary, we report that PI3K is necessary for the uptake of different amino acids stimulated with various growth factors. PI3K activity may therefore affect various aspects of VSMC functions through amino acid uptake [15].

## References

1. Douros A, Renoux C, Yin H, Filion KB, Suissa S, et al. (2017) Concomitant use of direct oral anticoagulants with antiplatelet agents and the risk of major bleeding in patients with nonvalvular atrial fibrillation *Am J Med* 132: 191-199.
2. Klopfer A (2021) Delayed global warming could reduce human exposure to cyclones. *Nature* 98:35.
3. Skagen FM, Aasheim ET (2020) Health personnel must combat global warming. *Tidsskr Nor Laegeforen* 14: 1-14.
4. Frolicher TL, Fischer E M, Gruber N (2018) Marine heatwaves under global warming. *Nature* 560: 360-364.
5. Kay J E (2020) Early climate models successfully predicted global warming. *Nature* 578: 45-46.
6. Traill LW, Lim LMM, Sodhi NS, Bradshaw CJA (2010) Mechanisms driving change: altered species interactions and ecosystem function through global warming. *J Anim Ecol* 79: 937-947.
7. Ross R (1986) The pathogenesis of atherosclerosis—an update. *New Eng J Med* 314: 488-500.
8. Duval C, Chinetti G, Trottein F, Fruchart JC, Staels B (2002) The role of PPARs in atherosclerosis. *Trends in molecular medicine* 8: 422-430.
9. Libby P, Ridker PM, Maseri A (2002) Inflammation and atherosclerosis. *Circulation* 105: 1135-1143.
10. Falk E (2006) Pathogenesis of atherosclerosis. *Exp Clin Cardiol* 47: C7-C12.
11. Hansson GK, Hermansson A (2011) The immune system in atherosclerosis. *Nat Immunol* 12: 204-212.
12. Italis A, Shantsila A, Proietti M, Kay M, Olshansky B, et al. (2021) Peripheral arterial disease in patients with atrial fibrillation: the AFFIRM study. *Am J Med* 134: 514-518.
13. Rothwell PM (2008) Prediction and prevention of stroke in patients with symptomatic carotid stenosis: the high-risk period and the high-risk patient. *Eur J Vasc Endovasc Surg* 35: 255-263.

14. Katsi V, Georgiopoulos G, Skafida A, Oikonomou D, Klettas D, et al. (2019) Non cardioembolic stroke in patients with atrial fibrillation. *Angiology* 70: 299-304.
15. Ugurlucan M, Akay MT, Erdinc I, Ozras DM, Conkbayir CE, et al. (2019) Anticoagulation strategy in patients with atrial fibrillation after carotid endarterectomy. *Acta Chir Belg* 119: 209-216.