**ROMANIAN ACADEMY**
**INSTITUTE OF CELLULAR BIOLOGY AND PATHOLOGY**
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**PhD THESIS**
*-BOOK SUMMARY-*

# The involvement of protein tyrosine phosphatases (PTPs) in intracellular signalling

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**CONTENTS**

**KEYWORDS**

Phosphatases

Kinases

Dephoshorylation

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Negative regulators

Vascular pathology

Insulin

Diabetes

Homeostasis

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Phosphatases are group of enzymes that dephoshorylate by removing a phosphate group from its substrate. They catalyse the hydrolysis of phosphoric acid monoesters in a phosphate ion and molecule with a free hydroxyl group. This action is directly opposite to that of phophorylases and kinases, which attach phosphate groups to their substrates by using energetic molecules like ATP.

Together with kinases, phosphatases regulate the phophorylation state of the cell. The balance between events controlled by these enzymes has an important role in intracellular signalling.

Since protein kinases (PKs) frequently phophorylates proteins substrates on tyrosine, serine and threonine residues, protein phophatases (PPs) exert the reversible effect engaging usually the same amino acids in proteins.

Protein tyrosine phosphatases (PTPs) are enzyme that removes phosphate groups from tyrosine residues in different proteins. Protein phophorylation on tyrosine residues (pTyr) is a common post-translational modification that can create novel recognition motifs for protein interactions and cell localization, affect protein stability and regulate enzyme activity. They can also be a key regulatory components in signal transduction pathways and they are very important in the control of cell growth, proliferation and differentiation.

PTPs are critical regulators of signal transduction under normal and pathophysiological conditions. These proteins exert a regulatory effect on signalling transduction at different levels by dephosphorylation of membrane receptors like protein tyrosine kinases (PTKs) and ionic channels. They can modulate signalling cascades by dephosphorylation of adaptor molecules, intracellular kinases and transcription factors. PTKs have been identified as targets for PTPs. Many PTPs dephosphorylates more than a PTK, and almost each PTK is dephosphorylated by more than a PTP.

Depending on substrate specificity, vascular PTPs contributes to vascular pathology. PTPs are critical regulators of tyrosine phosphorylation-dependent signalling events and may represent novel targets for therapeutic intervention in a variety of human diseases. PTPs are negative regulators of cell proliferation and mitogenic signalling cascades of Ang II, insulin, IGF-1, EGF and PDGF in several cell types, including vascular smooth muscle cells (VSMCs) (Marrero, 2004).

Because a critical regulatory step in insulin signal transduction is the dephosphorylation of signalling molecules by PTPs, it is plausible that enhanced activity of one or more PTPs could lead to insulin resistance (Choi et al., 2010).

The first part of my work „**The current state of knowledge**” presents PTPs throughout three chapters.

The first chapter presents classification and catalytic mechanism of phophatases. The balance between reactions exerted by phophatases and the one carried out by kinases seems to

be of the great importance in intracellular signalling. Phosphatases play a crucial role in homeostasis.

The second chapter describes PTPs classification and structure. Also, reveals some aspects regarding the substrate recognition and PTPs mechanism of action, as well as the main regulation processes for the action of PTPs.

In the last chapter it is shown the localisation of PTPs and their substrate molecules. This part of my work presents vascular wall-localised PTPs. Depending on cell type localisation, vascular PTPs regulate processes like angiogenesis, cell adhesion and motility. PTPs control signalling pathways involving growth factors and adhesion molecules. In this way PTPs have crucial role in vascular physiology and pathology. In the same chapter are presented PTPs that regulates insulin receptor signalling pathway. These enzymes play a substantial role in insulin signalling by reducing signalling and insulin receptor dephosphorylation. Along with diabetes mellitus there are also described some other pathologies in which PTPs play central roles. For PTPs activity, it is considerable the oxidation state of the enzymes, as well as the processes of oxidation/antioxidation developed at this level.

Our research, focused on finding new methods for PTPs activity manipulation, by using inhibitors and effects of oxidants/antioxidants on key molecules from insulin signalling pathway. Therefore in the second part of my work ''**Original contributions**", there have been two major objectives:

***1.*** Analysing the hyperglycaemia-induced changes in PTP-1B protein level as well as in protein level of the kinases involved in insulin signalling pathway. ***(chapter I, II si III)***

**1a**.The hydrogen peroxide and insulin action consequences.

**1b**. The effects of growth factor PDGF-BB stimulation.

*Experimental models*:

- „*in vitro*", smooth muscle cells isolated from human aorta, cell line
- „*in vivo*", streptozotocin injected Golden Syrian hamsters
- human biological material, from obese and obese-associated Type II diabetic patients

***2.*** Investigation of mechanisms by which oxidants/antioxidants and inhibitors act on PTP-1B and signalling kinases from insulin pathway ***(chapter I)***

**2a**. The role of high glucose (HG) and sodium orthovanadate.

**2b**. Modulation of molecules expression in hyperglycaemic conditions and PDGF-BB stimulation after catalase and superoxid dismutase action.

*Model experimental:*

- „*in vitro*", smooth muscle cells isolated from human aorta, cell line

**GENERAL DISCUSSIONS:**

Apprehending signalling pathways as well as molecules involved in vascular wall cell signalling, are critical for a better understanding of diabetes-associated endothelial dysfunctions.

In order to understand PTPs pathophysiology, structure and enzyme activity the studies conducted pursued two main objectives:

1. Protein level changes of the molecules from insulin signalling pathway induced by high glucose associated with growth factors and antioxidants stimulation ***(chapter I, II and III).***
2. The mechanisms by which antioxidants and inhibitors act on the molecules from insulin signalling pathway, reestablishing physiological function ***(chapter I).***

*„Original contributions" results presented over three chapters:*

1. High glucose, oxidative stress and insulin induce imbalance of insulin signalling molecules phosphorylation in SMCs. Hyperglycaemia determinates over expression of molecules (excepting activated Akt); oxidative stress has the same effect (less on PTP-1B); while insulin exerted antagonic effects on both molecules;

The originality of the study: ***I demonstrated that overexposure of SMCs to HG has a strong activating effect on Akt. ERK ½ was sensible to $H_2O_2$ , but not responsive to insulin action. Long-term exposure of SMCs to an even higher glucose concentration (i.e. 30 mM) leads to the occurrence of an additional band reactive with the PTP-1B antibody, of apparent molecular mass slightly higher than 51 kDa.***

2. Stimulation of SMCs with PDGF-BB determines an over expression of insulin signalling molecules. While PTP-1B protein expression is increased by PDGF-BB stimulation in a dose-dependent manner, pAkt and pERK ½ achieve a maximum value at 2 ng/ml.

The originality of the study consists of: ***stimulation with PDGF-BB of SMCs grown in high glucose, amplifies the PDGF-BB effect on insulin signalling molecules***.

3. Maintenance of SMCs in high glucose as well as in acute $H_2O_2$ stimulation, cause a diminished PTP activity duet to oxidation. $NaVO_4$, the PTPs nonspecific inhibitor, induces decrease of PTP-1B protein expression followed by molecular modification of pAkt and pERK ½.

The originality of the study : ***I established that part of endogenous oxidation (i.e. HG) and exogenous one (i.e. $H_2O_2$) responsible of PTPs reduced activation are reversible. In***

***hyperglycaemia, some PTP-1B enzyme oxidations are also reversible, capable of influencing catalytic activity of this phosphatase and regulation of insulin signalling pathway. In high glucose conditions, $NaVO_4$ leads to PTP-1B down regulation in SMCs.***

4. Overexposure of key molecules from insulin signalling pathway, following prolonged culture of SMCs in hyperglycaemic oxidant conditions, can be reversed by antioxidants. Catalase and superoxide dismutase are also able to re-establish protein levels of these molecules, increased due to oxidations as a consequence of PDGF-BB stimulation.

The originality of the study consists of: ***both antioxidants exercise strong effect on HG and PDGF-BB alone induced-oxidations, but especially on hyperglycaemia and PDGF-BB cumulative induced-oxidations.***

5. In streptozotocin injected Golden Syrian hamsters, diabetic hyperglycaemia imbalance the expression of phosphatases (i.e. PTP-1B) vs. kinases (i.e. $PI_3K$ and activated Akt) in the insulin signalling pathway. This disequilibrium is associated with endothelial pronounced permeability and SMCs apoptosis, and can also underline the aortic wall dysfunction as consequence of many changes in these cells.

The originality of the study consists of: ***demonstration of circulating hyperglycaemia effects on the balance between kinases / PTP-1B that acts in aortic wall, in the case of Type I diabetes.***

6. Obesity, as well as obesity associated with Type II diabetes influence protein expression of molecules associated with insulin signalling. In arterioles from subcutaneous adipose tissue, obesity induces a down regulation of insulin signalling molecules. This process is slowed in the case of obesity associated with Type II diabetes

The originality of the study consists of: ***I concluded that in both obese patients and obese associated with Type II diabetes patients, the arterioles from subcutaneous adipose tissue showed changes in expression of insulin signalling molecules important in both diseases***.

The imbalance in hyperglycaemia-induced phosphorylation/dephosphorylation events are accompanied by multiple reversible and irreversible oxidations which can interfere in diabetes -associated vascular dysfunction of aortic wall. Using PTPs antioxidants as well as the inhibitors could reverse changes occurred in smooth muscle cells or endothelial cells and that may improve vascular function. Therefore considering PTPs therapeutic targets would bring new elements in cardiovascular pathophysiology.

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