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BIOCHEMISTRY OF HYPERGLYCEMIA INDUCED VASCULAR DYSFUNCTION

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Abstract

Glucose enters the endothelium via a non-insulin sensitive GLUT-1 facilitated transporter that transports glucose continuously. Extracellular hyperglycemia is positively correlated with intracellular glucose. As the glucose levels increase, several changes in the glycolytic pathway, tricarboxilic acid cycle and the pentose phosphate pathway occur, all of them leading to an increase of oxygen reactive species (ROS). ROS are capable not only of directly impairing endothelial cells, but also indirectly by activating poly(ADP-ribose) polymerase, which inhibits glyceraldehyde 3-phosphate dehydrogenase. Further increase in glycloytic intermediates is followed by activation or overexpression of the main pathological pathways of hyperglycemia induced vascular damage: upregulation of glycation end-products, activation of protein kinase C, increased hexosamine pathway, and increased flux through polyol pathway, finally leading to the progressive narrowing and occlusion of the vessels.

key words: *endothelial dysfunction, hyperglycemia, oxygen reactive species, protein kinase C, hexosamine*

Basic Aspects of Glucose Metabolism

Providing adenosine triphosphate (ATP) for energy and carbon for biosynthesis reactions are the main roles of glucose metabolism. Glucose enters the endothelial cells through the non-insulin sensitive GLUT-1 facilitated transporter [1], further being phosphorylated to glucose-6-phosphate (G-6-P) by hexokinase at the expense of one molecule of ATP. There are three major pathways in glucose metabolism: glycolysis, tricarboxylic acid cycle (TCA cycle) and the pentose phosphate pathway (PPP).

Pentose phosphate pathway

Once synthesized, G-6-P either enters the PPP or continues its degradation through the glycolytic pathway. Entering the PPP, G-6-P is metabolized via two branches. The oxidative branch of PPP (with glucose-6-phosphogluconate dehydrogenase as the rate limiting enzyme) leads to ribulose-5-phosphate

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as the end product, the reactions yielding 2 molecules of nicotinamide adenine dinucleotide phosphate (NADPH). Next, during the nonoxidative branch catalyzed by transketolase and transaldolase, glyceraldehyde-3-P (G-3-P) and fructose-6-P (F-6-P) are produced. One possibility would be that F-6-P re-enters the PPP after being converted to G-6-P and the other would be that the two intermediates are pushed into the glycolytic pathway [2].

Glycolysis

F-6-P is the next metabolite of this pathway after G-6-P. Its phosphorylation to fructose-1,6-bisphosphate (F-1,6-P_2) by phosphofructokinase-1, the main regulatory enzyme, is an ATP-dependent reaction. Dihydroxyacetone-phosphate and G-3-P are generated by aldolase hydrolysis of F-1,6-P_2. During this reaction NADH is oxidized to NAD^+. As the final product of the glycolytic pathway, pyruvate either enters the TCA cycle or is converted to lactate. Under anaerobic conditions, due to the fact that NAD^+ regeneration is impaired, pyruvate is converted to lactate by lactate dehydrogenase (LDH), allowing NAD^+ regeneration essential for glycolysis to take place [2].

Tricarboxylic acid cycle

Under aerobic conditions pyruvate is carried into the mitochondrial matrix where it is metabolized to CO_2, H_2O, one molecule of reduced flavine adenine dinucleotide ($FADH_2$) and four molecules of NADH. Acetyl-CoA obtained from pyruvate condenses with oxaloacetate via citrate synthase to produce citrate. As for citrate, it continues its degradation as follows: α-ketoglutarate via aconitase, succinyl-CoA via α-ketoglutarate dehydrogenase complex, succinate via succinyl-CoA synthase, fumarate, and malate via fumarase. Malate may be converted to pyruvate by malic enzyme or to oxaloacetate via malate dehydrogenase.

The main role of TCA is production of NADH and $FADH_2$ that are essential for ATP synthesis via oxidative phosphorylation by the electron transport chain. Mitocondrial and cytosolic NADH transfers electrons to complex I (ubiquinone oxidoreductase), while $FADH_2$ gives them to complex II (succinate dehydrogenase) of the mithocondrial electron transport chain. The electrons are carried by coenzyme Q, transferred to cytochrome C and finally to molecular oxygen, which is reduced to H_2O in complex IV. A proton gradient is generated over the inner membrane. This gradient is used for ATP synthesis in complex V. Uncoupling proteins (UCPs), mitochondrial inner membrane proteins, can interfere with the process and keep the rate of ATP synthesis constant [3].

Basic Aspects of Metabolic Pathways in Hyperglycemia

It was proven that overproduction of superoxide (O_2^-) by the mitochondrial electron transport chain, and inhibition of glyceraldehyde-3 phosphate dehydrogenase (GAPDH), responsible for converting G-3-P to 1,3-diphosphoglycerate (1,3 DPG), are the keys of hyperglycemia induced endothelial damage [4,5]. The changes start once intracellular hyperglycemia leads to increase of pyruvate levels entering the TCA cycle, hence pushing more electron donors into the electron transport chain and increasing proton gradient across the inner mitochondrial membrane. Once the critical threshold is reached, electron transfer inside complex III is blocked and they are donated back to coenzyme Q and, subsequently, one at a time to molecular oxygen, producing superoxide and therefore upregulating reactive oxygen species (ROS) levels. In normoglycemic conditions superoxide dismutase degrades ROS to hydrogen peroxide, which in turn is converted to H_2O and O_2 [3,6].

The UCPs are a family of anion mitochondrial carriers that regulate proton transport, allowing dissipation of oxidation energy as heat [7]. Manganese superoxide dismutase (MnSOD) is a mitochondrial enzyme responsible for ROS degradation to hydrogen peroxide. Neither overproduction of ROS, nor inhibition of GAPDH takes place if they are prevented by UCPs or MnSOD. Otherwise, increased production of ROS in the mitochondria induces DNA strand breaks activating poly (ADP-ribose) polymerase (PARP). PARP is a DNA-repair enzyme that normally lies inactive in the nucleus. If activated by DNA damage, it splits NAD^+ into ADP-ribose and nicotinamide mononucleotide. Further it proceeds to make polymers of ADP ribose, catalyzing GAPDH poly(ADP-ribosyl)ation and thus inactivating this enzyme [8]. Usually GAPDH lies in the cytosol, but during cell death it translocates in the nucleus [9]. Hence, the glycolytic pathway is compromised leading to an increased number of intermediates that are upstream of GAPDH. An increase in glyceraldehide-3-phosphate (G-3-P) levels upregulates advanced glycation end-products (AGEs) through methylglycoxal, and protein kinase C (PKC) through diacylglycerol. Increased level of fructose-6-phospahate is diverted into the hexosamine pathway, while increased glucose stimulates flux through the polyol pathway, where the enzyme aldose reductase reduces it, consuming NADPH in the process [3].

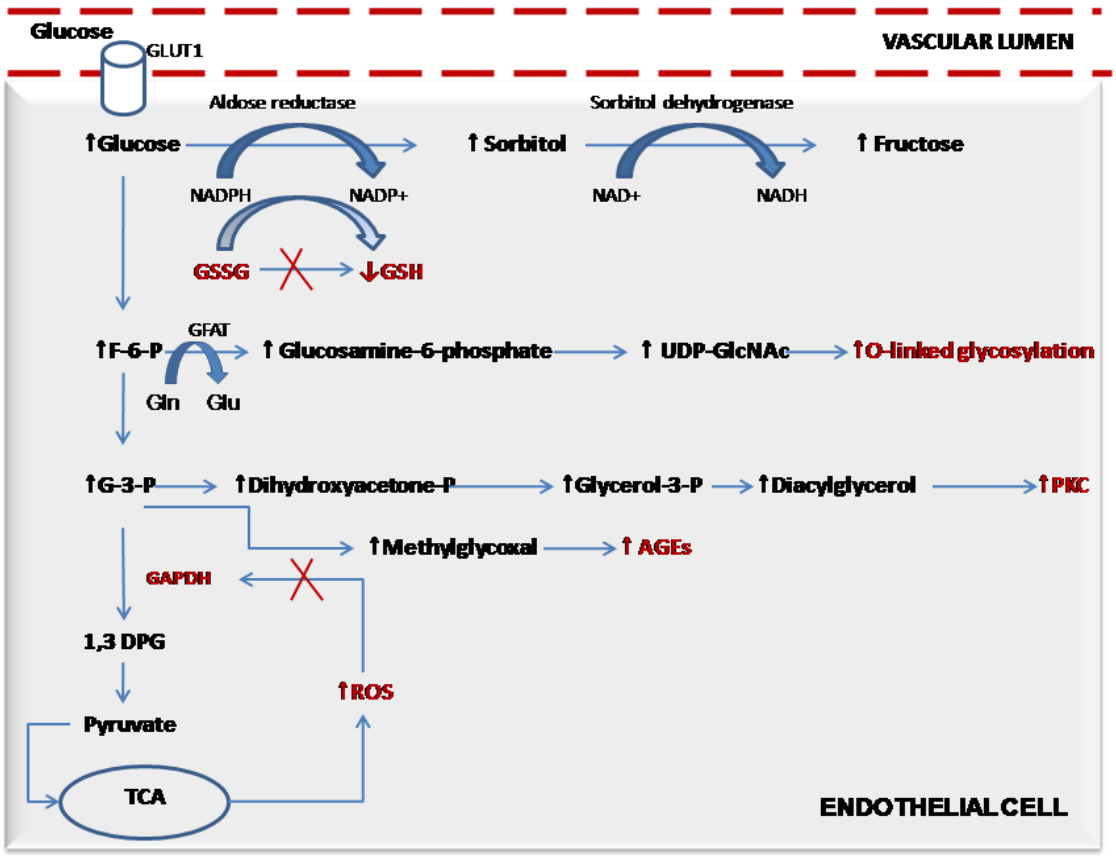

Figure 1. *Metabolic Pathways in Hyperglycemia*
GSSG = oxidized glutathione; GSH = reduced glutathione;
UDP-GlcNAc = uridine diphosphate N-acetylglucosamine;
GFAT = glutamine fructose-6-phosphate amidotransferase.

Increased Production of AGE

Production of AGEs is correlated with the degree of hyperglycemia and oxidative stress, but also with the rate of protein glycoxidation. Intracellular AGEs can arise from the autooxidation of glucose to glyoxal, the decomposition of an Amadori product to 3–deoxyglucosone, or methylglyoxal synthesis from glyceraldehyde-3-phosphates, known as α-dicarbonyls or oxoaldehydes. The major AGEs is methylglyoxal [10]. AGEs interact with amino, sulfhydryl, and guanidine functional groups in proteins altering their properties. Therefore AGEs impair cellular functions by modifying intracellular proteins, extracellular matrix components, and also altering plasma proteins [6].

Consequences: AGEs formed on extracellular components such as collagen, laminin, elastin, and vitronectin impair constitution of the matrix and increase stiffness of the vessels. AGEs formed on plasma proteins interact with AGE receptors on cells such as macrophages, vascular endothelial cells, and vascular smooth muscle cells, promoting ROS production, upregulation of nuclear factor-kappaB (NF-kB), thrombomodulin, tissue growth factor and vascular cell adhesion molecule-1 (VCAM-1) with procoagulatory and proinflammatory consequences [11]. As for intracellular AGEs, they change cellular properties that are critical in vascular homeostasis, increasing expression of angiopoietin-2 (Ang-2), thus producing endothelial damage through intracellular adhesion molecule-1 (ICAM-1), VCAM-1 and tumor necrosis factor α (TNF - α) [12].

Activation of PKC

Member of the cAMP-dependent protein kinase/protein kinase G/protein kinase C (AGC) family, PKC is a serine/threonine-related protein kinase with an important regulatory role in different cellular responses. There are eleven isoforms of this enzyme [12]. The conventional isoforms, PKC-α, -β1, -β2, and –γ, are activated by diacylglycerol, calcium, phosphatidylserine and phorbol esters, while the novel isoforms, PKC-δ, -ε, -θ and -η are activate by all the above mentioned substrates except for calcium. Instead the atypical isoforms, PKC-ζ and -ι/λ, are calcium and phospholipids independent. Oxidants such as H_2O_2 and mitochondrial superoxide, induced by hyperglycemia may activate them [13].

Mainly the PKC-β and PKC-ε are associated with vascular dysfunction in hyperglycemic patients. PKC activation is possible due to the de novo synthesis of diacylglycerol [6]. Excessive production of G-3-P, followed by dihydroxyacetone phosphate production with further reduction and acylation of this product enhances diacylglycerol levels, thus activating PKC [13]. There is evidence suggesting that interaction between AGEs and their cell surface receptors stimulate PKC activity [12].

An important fact is the existence of an increase in PKC activity not only during prolonged hyperglycemic conditions but also after a short term exposure. Clinical studies have proven a rapid increase in PKC expression after 1h of exposure to hyperglycemia. This rapid change may account for the reperfusion injury in patients having a stroke [14].

Consequences: PKC has a great impact on endothelium-dependent nitric oxide-mediated dilatation. Increased expression of NAD(P)H oxidase is responsible for ROS production, reflecting the pro-constrictive role of the enzyme. PKC mediates the reaction between nitric oxide (NO) and superoxide, peroxynitrite being the final product. Not only that NO bioavailability decreases and endothelial vasodilatation is impaired, but peroxynitrite formation may also affect cerebrovascular

reactivity by inhibiting prostacyclin synthase (causing prostaglandin H2 accumulation - substrate for tromboxane A2) and calcium-dependent K^+ channel (shown to cause constriction of cerebral vessels in animals) [15]. Another PKC mediated mechanism that enhances vascular dysfunction includes endothelial nitric oxide synthase (eNOS) dephosphorylation at Ser1177 and phosphorylation at Thr495 [16]. As NO level falls down, NF-kB is activated. During acute hyperglycemia its level reach a peak in only 4 hours, promoting inflammation through leukocyte adhesion, mediated by VCAM-1, E-selectin, ICAM-1 [17]. Perfusion is also decreased by plugging and fibrin deposition, secondary to overexpression of the plasminogen activator inhibitor 1 (PAI-1), a fibrinolytic inhibitor [6].

The Hexosamine Pathway

Once entering the cell, 2% to 5% of the total glucose is metabolized via the hexosamine pathway. F-6-P diverted from glycolysis into this pathway is used for providing substrates for UDP-GlcNAc. Glucose and glucosamine are the main substrates. Once glucose is converted to F-6-P,L-glutamine-D-fructose 6-phosphate amido-transferase (GFAT) (the rate limiting enzyme) converts it to glucosamine-6-phosphate, with glutamine being the amine donor. On the other hand, glucosamine entering the endothelial cells via the glucose transporter system may rapidly increase glucosamine-6-phosphate level, due to its phosphorylation mediated by hekokinase and thus bypassing GFAT. The final product of the pathway (UDP-GlcNAc) is important for the synthesis of glycolipids, glycoproteins and proteoglycans [18]. Also it is responsible for O-linked N-acetylglucosamination of nucleo-cytoplasmic proteins via O-GlcNAc transferase. β-N-acetylglucosaminidase (O-GlcNAcase) has also an important role in the process of glycation. The O-linked enzymatic attachment of N-acetyl-glucosamine on serine and threonine residues of nuclear and cytoplasm proteins is responsible for modulating signaling, and influencing protein expression, trafficking and degradation [19]. The main consequences are increased modification of the transcription factor Sp1 resulting in overexpression of transforming growth factor-β1 and PAI-1, and down-regulation of NO production due to O-acetylglucosamination of eNOS at the Akt activation site [6].

Consequences: O-GlcNAcylation of endothelial nitric oxide synthase (eNOS)-the primary source of NO-impairs the following processes: vasodilatation via guanylate cyclase pathway and inhibition of plaque formation by blocking platelet-leukocyte interactions with vascular wall and by preventing smooth muscle cell migration and proliferation, and also inhibits activation of NF-kB [20,21].

Increased Flux through the Polyol Pathway

During normoglycemic conditions, aldose reductase reduces toxic aldehydes (generated by ROS in the cell) to inactive alcohols. It is found in tissues such as vascular cells, nerve, retina, glomerulus, lens, where glucose transport is not insulin sensitive [22]. Therefore hyperglycemia leads to increased intracellular glucose levels causing glucose reduction to sorbitol by aldose reductase, with NADPH as a cofactor. Sorbitol is instead oxidized to fructose by sorbitol dehydrogenase, with NAD^+ being reduced to NADH [12].

Consequences: It was proven that this pathway causes significant damage to the nerves [23] and to the lenses [24]. Firstly, NADPH is the essential cofactor required for GSH regeneration from GSSG. Therefore, being consumed in the polyol pathway causes increased susceptibility to oxidative stress [25].

Secondly, intense flux through the polyol pathway perturbs the intracellular levels of osmolytes, such as sorbitol, myo-inositol, taurine. In diabetic nerves, in order to maintain the osmotic equilibrium, excessive accumulation of sorbitol is accompanied by a drop in the myo-inositol content. Myo-inositol depletion will impair phosphoinositide metabolism, limiting diacylglycerols synthesis known to modulate Na^+/K^+-ATPase activity. Finally, this will result in nerve conduction abnormalities [26].

Conclusions

Exposure to abnormally high glucose concentrations causes an activation of glycation end-products generation, activation of protein kinase C, increased hexosamine pathway, and increased flux through polyol pathway. These four mechanisms are triggered by the excessive mitochondrial production of superoxide and are causing damage to cells that are unable to control the rate of trans-membrane glucose transport. Endothelial cells are major target of hyperglycemia induced damage, leading to pro-constrictive, pro-thrombotic and pro-inflammatory effects likely to cause or enhance cerebrovascular and cardiovascular disease.

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