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Altered energy state reversibly controls smooth muscle contractile function in human saphenous vein during acute hypoxia–reoxygenation: Role of glycogen, AMP-activated protein kinase, and insulin-independent glucose uptake

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ABSTRACT

Hypoxia is known to promote vasodilation of coronary vessels through several mediators including cardiac-derived adenosine and endothelium-derived prostanoids and nitric oxide. To date, the impact of endogenous glycogen depletion in vascular smooth muscle and the resultant alterations in cellular energy state (*e.g.*, AMP-activated protein kinase, AMPK) on the contractile response to G protein-coupled receptor agonists (*e.g.*, serotonin, 5-HT) has not yet been studied. In the present study, *ex vivo* exposure of endothelium-denuded human saphenous vein rings to hypoxic and glucose-deprived conditions during KCl-induced contractions for 30 min resulted in a marked depletion of endogenous glycogen by ~80% (from ~1.78 μmol/g under normoxia to ~0.36 μmol/g under hypoxia). Importantly, glycogen-depleted HSV rings, which were maintained under hypoxia/reoxygenation and glucose-deprived conditions, exhibited significant increases in basal AMPK phosphorylation (~6-fold ↑) and 5-HT-induced AMPK phosphorylation (~19-fold ↑) with an accompanying suppression of 5-HT-induced maximal contractile response (~68% ↓), compared with respective controls. Exposure of glycogen-depleted HSV rings to exogenous D-glucose, but not the inactive glucose analogs, prevented the exaggerated increase in 5-HT-induced AMPK phosphorylation and restored 5-HT-induced maximal contractile response. In addition, the ability of exogenous D-glucose to rescue cellular stress and impaired contractile function occurred through GLUT1-mediated but insulin/GLUT4-independent mechanisms. Together, the present findings from clinically-relevant human saphenous vein suggest that the loss of endogenous glycogen in vascular smooth muscle and the resultant accentuation of AMPK phosphorylation by GPCR agonists may constitute a yet another mechanism of metabolic vasodilation of coronary vessels in ischemic heart disease.

1. Introduction

Glycogen, a principal storage form of carbohydrate, serves as a readily mobilizable source of energy to control vital functions in striated muscles (*e.g.*, skeletal muscle and myocardium) and vascular smooth muscle even under anaerobic conditions [1–4]. Dysregulation of glycogen turnover manifests in skeletal muscle and cardiovascular abnormalities, and it results from deficiency of glycogen-metabolizing enzymes [5] or mutation of AMP-activated protein kinase $\gamma_{2/3}$ (AMPK $\gamma_{2/3}$) subunit [6,7]. For instance, in patients with inherited deficiency of lysosomal acid α-glucosidase, failure of glycogen breakdown results in lysosomal glycogen storage disease (type II, Pompe disease), which is characterized by muscular hypotonia, cardiomegaly, aortic/basilar artery aneurysm,

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and aortic stiffness [8–10]. In patients with naturally occurring mutations in AMPKγ_2 subunit (encoded by *PRKAG2* gene), dysregulated metabolism results in cytoplasmic glycogen accumulation and hypertrophic cardiomyopathy [7]. While the genetic defects in these intracellular enzymes culminate in glycogen storage disease phenotype, metabolic stresses such as hypoxia and contraction promote glycogen depletion in striated and smooth muscles [2,4,11,12]. Studies with striated muscles have revealed the impact of glycogen depletion toward ATP loss, AMPK activation, and altered functional response [11,12]. Nevertheless, the relationship between glycogen content and AMPK activation and their regulatory roles toward smooth muscle contraction have thus far not been examined in vascular beds from any species, including the conduits used in patients undergoing coronary artery bypass grafting (CABG) surgery, under normoxic or hypoxic conditions.

Endogenous glycogen serves as an 'energy reserve' for vascular smooth muscle contraction [2,3]. Seminal studies by several investigators over the past three decades have provided compelling evidence for active glycogen turnover (glycogen synthesis and its degradation) in vascular smooth muscle using porcine carotid artery as a model system [2,3,13,14]. The metabolic pathways such as glycogenolysis and glycolysis contribute to the formation of ATP, an 'immediate energy source' for contracting smooth muscle. Of importance, hypoxic conditions result in a marked depletion of glycogen in arterial smooth muscle [2,4,15]. While glycogen depletion is known to decrease 'cellular energy state' (↓ATP) in vascular smooth muscle [4], it remains unknown as to how this will influence the activation state of AMPK ('an energy sensor') during agonist-induced contractions. Notably, AMPK functions as a sensor of cellular energy reserve (glycogen content) and cellular energy state (AMP/ATP or ADP/ATP ratio) [16,17]. This is evident from the structural features of heterotrimeric AMPK complexes that comprise a catalytic subunit (α) and two regulatory subunits (β and γ). AMPKβ subunit contains a glycogen-binding domain, the association of which to glycogen results in AMPK inhibition [16,18]. Stress-induced glycogen depletion and ATP loss would promote AMPK activation. The increase in AMPK activity occurs due to the replacement of ATP on AMPKγ subunit by ADP and AMP, and the consequent increase in AMPKα phosphorylation (at Thr^{172} residue) and allosteric activation [17]. Thus, it is important to determine the critical relationship between glycogen content and AMPK activation in intact vascular smooth muscle during agonist-induced contractions under normoxic and hypoxic conditions.

Under metabolic stress, exogenous glucose (an energy substrate) has been shown to suppress AMPK activation in striated muscles [19,20]. The uptake of glucose in VSMCs occurs through plasma membrane-localized glucose transporter-1 (GLUT1) or insulin-dependent GLUT4-mediated mechanism [21–24]. While glucose uptake and glycolysis would promote ATP formation to diminish AMPK activity, the interplay between endogenous glycogen and exogenous glucose toward altered AMPK phosphorylation remains to be examined in vascular smooth muscle during agonist-induced contraction.

Recently, we and several other investigators have shown that metabolic stress [25], metformin [26], and AICAR [26–28] inhibits agonist-induced smooth muscle contraction through AMPK activation in endothelium-denuded arterial ring preparations from rodent or porcine models. In addition, previous studies have shown that serotonin, a platelet-derived vasoactive agonist, dilates coronary resistance vessels but induces constriction of coronary conduit vessels (cited in [29]). In the present study, we hypothesize that in saphenous vein conduits from human subjects, glycogen depletion by acute hypoxia activates AMPK thereby compromising serotonin-induced smooth muscle contraction. The objectives of the present study are to determine: (i) the effects of hypoxia on glycogen content, AMPK phosphorylation, and serotonin-induced smooth muscle contraction; and (ii) the effects of hypoxia-reoxygenation on basal and serotonin-induced changes in AMPK phosphorylation and contraction in the absence or presence of exogenous glucose during isometric contractions in human saphenous vein.

2. Materials and methods

2.1. Materials

Serotonin (5-hydroxytryptamine hydrochloride), phenylephrine hydrochloride, acetylcholine chloride, D-glucose, L-glucose, and 2-deoxy-D-glucose were purchased from Sigma–Aldrich (St. Louis, MO). The primary antibodies for AMPKα, phospho-AMPKα^{Thr172}, ERK1/2, phospho-ERK1/2, Akt, and phospho-Akt^{Ser473} were purchased from Cell Signaling Technology (Danvers, MA). The primary antibodies for GLUT1, GLUT4, and β-actin were purchased from Abcam (Cambridge, MA). HRP-conjugated goat anti-rabbit secondary antibody was purchased from Bio-Rad (Hercules, CA). All other chemicals were from Fisher Scientific (Fair Lawn, NJ) or Sigma–Aldrich (St. Louis, MO).

2.2. Human subjects and saphenous vein specimens

The study protocol was approved by the Institutional Review Board at the Georgia Regents University, Charlie Norwood VA Medical Center, Penn State Hershey College of Medicine, and University of Georgia. According to the approved protocol, the leftover saphenous vein specimens from subjects undergoing coronary artery bypass grafting (CABG) surgery were used for the current study. Saphenous vein specimens, which were obtained from a total of 23 subjects, were transported from the operating room to the laboratory in ice-cold Krebs buffer. The specimens from subjects with a history of diabetes were excluded in this study.

2.3. Animals and isolation of thoracic aorta

All animal experiments were performed in accordance with the Charlie Norwood Veterans Affairs Medical Center Institutional Animal Care and Use Committee guidelines, and were approved by the committee. Adult male Wistar rats (Charles River Laboratories, Wilmington, MA) were maintained in a room at a controlled temperature of 23 °C with a 12:12-h dark-light cycle. They had free access to water and standard rodent chow diet. On the day of contractility studies, rats were sacrificed followed by isolation of thoracic aorta as described [26].

2.4. Preparation of human saphenous vein rings and isometric tension measurements

Human saphenous vein (HSV) specimens were immediately placed in a petri-dish containing ice-cold oxygenated Krebs–Henseleit bicarbonate (KHB) buffer (118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, and 11 mM glucose; pH 7.4). HSV was carefully cleaned free of any adherent fat and connective tissue. Endothelium was removed by gently rubbing the luminal surface using a polyethylene tube. HSV was then cut into 2-mm rings and mounted in the organ bath system for isometric tension measurements, as described previously [26].

After passing the stainless steel wires through the lumen, HSV ring preparations were suspended from the isometric force displacement transducers (Model FT03; Grass Technologies, West Warwick, RI) and kept immersed in 10 ml of KHB buffer in the organ bath system (8 chambers, Radnoti Glass Technology, Monrovia, CA). The KHB buffer was bubbled continuously with a