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The hypotensive effect of acute and chronic AMP-activated protein kinase activation in normal and hyperlipidemic mice

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ABSTRACT

AMP-activated protein kinase (AMPK) is present in the arterial wall and is activated in response to cellular stressors that raise AMP relative to ADP/ATP. Activation of AMPK in vivo lowers blood pressure but the influence of hyperlipidemia on this response has not been studied. ApoE$^{-/-}$ mice on high fat diet for 6 weeks and age-matched controls were treated with the AMPK activator, AICAR daily for two weeks. Under anesthesia, the carotid artery was cannulated for blood pressure measurements. Aortic tissue was removed for in vitro functional experiments and AMPK activity was measured in artery homogenates by Western blotting. ApoE$^{-/-}$ mice had significantly raised mean arterial pressure; chronic AICAR treatment normalized this but had no effect in normolipidemic mice, whereas acute administration of AICAR lowered mean arterial pressure in both groups. Chronic AICAR treatment increased phosphorylation of AMPK and its downstream target acetyl-CoA carboxylase in normolipidemic but not ApoE$^{-/-}$ mice. In aortic rings, AMPK activation induced vasodilation and an anticontractile effect, which was attenuated in ApoE$^{-/-}$ mice. This study demonstrates that hyperlipidemia dysregulates the AMPK pathway in the arterial wall but this effect can be reversed by AMPK activation, possibly through improving vessel compliance.

1. Introduction

AMP-activated protein kinase (AMPK) is an enzyme with a central role in cellular energy homeostasis that is activated in response to a change in the cellular balance of AMP to ADP/ATP. Often described as the cell's fuel gauge, it becomes activated in response to cellular stressors including inflammation, hypoxia and oxidant stress [1]. Recent evidence (reviewed in [2]) points to a vasculoprotective role for AMPK activation that may be mediated through inducing endothelial NO production [3,4], preventing EC-monocyte adhesion [5] and positively regulating vascular redox balance via upregulating expression of manganese superoxide dismutase [6], and reducing reactive oxygen species generation in response to hyperglycemia [7]. AMPK can also reduce the proliferative effects of stimuli such as platelet derived growth factor and angiotensin II (Ang-II) [8], and is likely to be intimately involved in vascular remodeling [9].

AMPK is a trimer of α (catalytic) and β and γ (regulatory) subunits which, although ubiquitous, has tissue-specific subunit isoform expression. In vascular cells, isoforms containing the α_1 subunit predominate [10] while α_2 predominates in cardiac tissue [11]. AMPK activation involves phosphorylation of Thr^{172} on the α subunit by upstream AMPK kinases (AMPKK), primarily LKB-1 [12] and CaMKKβ [13]. Activated AMPK phosphorylates several downstream targets, including acetyl-coenzyme A carboxylase (ACC) [14]. At a cellular level, this stimulates fatty acid oxidation, mitochondrial biogenesis and glucose uptake, inhibition of fatty acid synthesis, cholesterol production and gluconeogenesis [2]. In atherosclerosis, the presence of oxidized low density lipoproteins increases endoplasmic reticulum (ER) stress [15] and causes a 40-fold increase in expression of protein phosphatase 2A (PP2a), the enzyme responsible for inactivating AMPK [16]. Recent evidence suggests that activation of AMPK in atherosclerosis has beneficial effects including reversing ER stress [15] and stimulating reverse cholesterol transport from foam cells to reduce plaque area in mice deficient in apolipoprotein E (ApoE$^{-/-}$) [17,18].

Hypertension is a risk factor in development of atherosclerosis and dysfunction of the endothelium may be a feature common to both pathologies [19]. The ApoE$^{-/-}$ mouse develops spontaneous hypercholesterolemia and atherosclerosis with the first signs of disease occurring at 6 to 8 weeks with features accelerated by high fat feeding [20,21]. Some studies [22] have measured an increased blood pressure in ApoE$^{-/-}$ mice while others suggest no difference from control, non-atherosclerotic mice [23,24]. Previous studies have shown that acute administration of the AMPK activating agent, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) reduces mean arterial blood pressure (MAP)

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in both rodents and humans [25,26]. Furthermore, spontaneously hypertensive rats dosed with AICAR showed an acute drop in MAP that was not seen in control WKY rats, suggesting that AMPK could play a role in reducing hypertension [27]. Long-term administration of AICAR or resveratrol, another activator of AMPK, also reduced blood pressure in obese Zucker rats [28,29] and Ang-II-induced hypertensive mice [30]. In vitro experiments using aortae from mice lacking AMPKα1 indicate that AMPK may improve endothelial function via endothelial nitric oxide synthase (eNOS) phosphorylation [31], while chronic activation of AMPK in mice reversed the deleterious effects of the vasoconstrictor 20-HETE on eNOS [32]. Collectively, these studies suggest that activation of AMPK may reduce blood pressure though an effect on vascular eNOS. However, what is not clear is how hyperlipidemia or established fibrofatty plaques affect AMPK activity within the arterial tree and if this attenuates the ability of AMPK activation to modulate blood pressure. Consequently, the aims of this study were to assess the effect of high fat feeding on MAP in ApoE$^{-/-}$ mice and whether chronic activation of AMPK in vivo affects blood pressure and vascular AMPK activity. A further aim was to study how the presence of atherosclerotic lesions affects the hypotensive response to acute AMPK activation and the vasodilator response to AMPK activating agents in vitro.

2. Methods

2.1. Animal models

All in vivo experiments were performed in accordance with the United Kingdom Animals (Scientific Procedure) Act of 1986. Mice were housed at the University of Glasgow and maintained on 12 hour cycles of light and dark and at ambient temperature. Two strains of mice were used: ApoE$^{-/-}$ (bred in-house) and the genetic background control (C57BL/6, Harlan). In all experiments, age-matched male mice were used. C57BL/6 mice were fed a standard chow diet while ApoE$^{-/-}$ mice commenced a high fat diet (21% lard and 0.15% cholesterol, SDS) at 8 weeks of age. High fat diet was continued for either six weeks to induce hyperlipidemia without arterial lesions or for 12 weeks, a time point at which we have previously demonstrated that atherosclerotic lesions are present in the arterial tree [33]. At the end of the experimental protocol, mice were used to study the effects of acute or chronic AMPK activation on blood pressure and vascular function as outlined below. For some of the vascular function experiments, mice deficient in the main vascular isoform of AMPK, α1 (AMPKα$^{-/-}$) and their wild-type littermates (S129 strain) were used. These were originally obtained from Professor Benoit Viollet (Institut Cochin, Paris, France), bred in-house and age-matched to the 12 week fat-fed ApoE$^{-/-}$ mice.

2.2. In vivo hemodynamic measurements

To study the effect of chronic AMPK activation on blood pressure and the effect of hyperlipidemia, ApoE$^{-/-}$ mice (on diet for 4 weeks) were administered daily i.p. injections of the AMPK activating agent, AICAR (Toronto Research Chemicals Inc.) or an equivalent volume of vehicle (distilled water) at a dose of 400 mg/kg [34,35]. An age-matched group of C57BL/6 mice was also treated concurrently with either AICAR or vehicle. Weight was monitored throughout to allow AICAR dose adjustment if required. For all groups, treatment continued for 14 days at which time hemodynamic measurements were performed under terminal anesthesia. At the end of the procedure, blood was removed by cardiac puncture into EDTA-containing Vacutainers for measurement of plasma myeloperoxidase (MPO), spleen, heart and liver were also removed and weighed. The carotid artery and aorta were dissected out, cleaned of surrounding fat and connective tissue and snap-frozen in liquid nitrogen for later analysis. To study the effect of acute AICAR administration on blood pressure in C57BL/6 mice and ApoE$^{-/-}$ mice with established atherosclerosis following 12 weeks of high fat diet, mice were injected with a single dose of AICAR (400 mg/kg i.p.) or vehicle 45 min before induction of terminal anesthesia and recording of blood pressure.

Mice were anesthetized using an isoflurane mixture and a polyurethane cannula (Harvard Apparatus) filled with heparinized saline was inserted into the carotid artery. The cannula was connected to an Elcomatic E751A pressure transducer and MP35 data acquisition system (BIOPAC Systems Inc.). The recordings were made using commercially available software and data was acquired for at least 10 min in each animal. MAP was derived from analysis of at least 5 points within the 10 minute analysis period.

2.3. Western blotting

Aortae were pulverized in liquid nitrogen and re-suspended in ice-cold cell lysis buffer (50 mM Tris pH 7.4, 50 mM NaF, 1 mM Na_4PPi, 1 mM EGTA, 1 mM EDTA, 1% Triton X-100, 1 mM DTT and 1% cocktail of protease inhibitors). All the samples (10 μg) were run on NuPAGE Novex 4–12% Bis-Tris mini gels (Life Technologies), transferred to nitrocellulose membrane and analyzed with the following primary antibodies: AMPKα (1:1000; Cell Signaling Technology), phospho-AMPKα (1:1000; Cell Signaling Technology), ACC (1:1000; Cell Signaling Technology), phospho-ACC (1:1000; Cell Signaling Technology), GAPDH (1:40000; Abcam), and α tubulin (1:5000; Abcam). Protein bands were visualized using an enhanced chemiluminescence detection kit (Thermo Scientific) and the density was quantified using a GS-800™ Calibrated Densitometer (BioRad) and Quantity One BioRad software.

2.4. Measurement of MPO and plasma lipids

The MPO content of plasma from C57BL/6 and ApoE$^{-/-}$ mice was analyzed using a mouse MPO ELISA kit (Hycult® Biotech Inc.). Plasma samples were diluted 1 in 16 in dilution buffer and the assay was performed as per the manufacturer's instructions. Absorbance was measured spectrophotometrically at 450 nm using a SpectraMax M2 microplate reader. Plasma total cholesterol, HDL cholesterol and triglycerides were measured in undiluted plasma on an ILAB 600 clinical chemistry analyzer, using Roche kits for HDL cholesterol. Triglyceride and cholesterol kits were from Randox Laboratories. All values were calibrated using the assigned kit calibrators and checked against the relevant quality controls.

2.5. Small vessel wire myography

To assess the effects of AMPK activation on vascular tone in atherosclerotic mice and mice treated with AICAR, mouse aorta was removed, cleaned of all fat and connective tissue and cut into 2 mm rings. In most experiments the endothelium was removed by gently rubbing the interior of the vessel with a human hair and removal confirmed by lack of (<10%) vasodilator response to 10^{-6} M acetylcholine. Artery rings were mounted on two stainless steel wires in a four channel wire myograph (Danish Myo Technology), set to an optimum tension of 9.8 mN [36] and allowed to equilibrate for at least 30 min before use. Vessels were bathed in Krebs–Henseleit buffer (118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.03 mM KH_2PO_4, 11 mM glucose and 2.5 mM $CaCl_2$) at 37 °C and gassed continuously with 95% O_2 and 5% CO_2. Vessel viability was checked with 40 mM KCl and preconstriction was produced by addition of a submaximal concentration (3×10^{-8} M) of the thromboxane A_2 mimetic, U46619. Cumulative concentration–response curves to AICAR (10^{-4} M–10^{-2} M) or AMPK activator A769662 (10^{-6} M–5×10^{-4} M) were performed with addition of drug at 10 minute intervals. For all experiments, data were expressed as a percentage of relaxation of the U46619-induced tone. We also measured the effect of preincubation of AMPK activating agents on the constrictor response to U46619. In this case, 2 mM AICAR or 30 μM A769662 was added 45 min prior to U46619 contraction.