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Disclosing caffeine action on insulin sensitivity: Effects on rat skeletal muscle

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ABSTRACT

Caffeine, a non-selective adenosine antagonist, has distinct effects on insulin sensitivity when applied acutely or chronically. Herein, we investigated the involvement of adenosine receptors on insulin resistance induced by single-dose caffeine administration. Additionally, the mechanism behind adenosine receptor-mediated caffeine effects in skeletal muscle was assessed.

The effect of the administration of caffeine, 8-cycle-1,3-dipropylxanthine (DPCPX, A_1 antagonist), 2-(2-Furanyl)-7-(2-phenylethyl)-7H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidin-5-amine (SCH58261, A_{2A} antagonist) and 8-(4-{[(4-cyanophenyl)carbamoylmethyl]-oxy}phenyl)-1,3-di(n-propyl)xanthine (MRS1754, A_{2B} antagonist) on whole-body insulin sensitivity was tested. Skeletal muscle Glut4,5′-AMP activated protein kinase (AMPK) and adenosine receptor protein expression were also assessed. The effect of A_1 and A_{2B} adenosine agonists on skeletal muscle glucose uptake was evaluated *in vitro*. Sodium nitroprussiate (SNP, 10 nM), a nitric oxide (NO) donor, was used to evaluate the effect of NO on insulin resistance induced by adenosine antagonists.

Acute caffeine decreased insulin sensitivity in a concentration dependent manner (E_{max} = 55.54 ± 5.37%, IC_{50} = 11.61 nM), an effect that was mediated by A_1 and A_{2B} adenosine receptors. Additionally, acute caffeine administration significantly decreased Glut4, but not AMPK expression, in skeletal muscle. We found that A_1, but not A_{2B} agonists increased glucose uptake in skeletal muscle. SNP partially reversed DPCPX and MRS1754 induced-insulin resistance.

Our results suggest that insulin resistance induced by acute caffeine administration is mediated by A_1 and A_{2B} adenosine receptors. Both Glut4 and NO seem to be downstream effectors involved in insulin resistance induced by acute caffeine.

1. Introduction

Caffeine is the behaviorally active substance most widely consumed in the world (Fredholm et al., 1999). When used regularly, this xanthine appears to have minor negative consequences on human health. In fact, several epidemiological studies reported beneficial effects of long-term caffeine intake on type 2 diabetes and metabolic syndrome risk (van Dam and Feskens, 2002; van Dam et al., 2004). Recently, our group demonstrated that chronic caffeine intake prevents the development of insulin resistance (IR) in diet-induced IR rats, an effect that is related with a decrease in sympathetic nervous system activity (Conde et al., 2012). This sensitizer effect of chronic caffeine intake contrasts with the consequence of acute caffeine administration that has been associated with an increased IR (Greer et al., 2001; Keijzers et al., 2002). At the cellular level, caffeine acts through 3 distinct mechanisms: the inhibition of cyclic nucleotide phosphodiesterases, the increase in intracellular calcium and by adenosine

Abbreviations: AMPK, 5′-AMP activated protein kinase; Bay-60-6583, 2-[[6-Amino-3,5-dicyano-4-[4-(cyclopropylmethoxy)phenyl]-2-pyridinyl]thio]-acetamide; CPA, N^6-Cyclopentyladenosine; DPCPX, 8-cycle-1,3-dipropylxanthine; GPCR, G protein-coupled receptors; IR, insulin resistance; ITT, insulin tolerance test; K_{ITT}, constant of the insulin tolerance test; MRS1754, 8-(4-{[(4-cyanophenyl)carbamoylmethyl]-oxy}phenyl)-1,3-di(n-propyl)xanthine; NO, nitric oxide; RTK, receptor tyrosine kinases; SCH58261, 2-(2-Furanyl)-7-(2-phenylethyl)-7H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidin-5-amine; SNP, sodium nitroprussiate.

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receptors inhibition, being this the only mechanism significantly affected by standard doses of human caffeine consumption (Fredholm et al., 1999).

Adenosine is an ubiquitous mediator that acts through the activation of 4 different types of adenosine receptors coupled to G proteins, A_1, A_{2A}, A_{2B} and A_3 (Conde et al., 2009). It is known that adenosine regulates several physiological processes however, its role in glucose homeostasis and insulin sensitivity is still controversial (Burnstock and Novak, 2013). In adipose tissue adenosine inhibits lipolysis (Johansson et al., 2008), increases insulin-stimulated glucose transport and augments the number of Glut4 transporters in the plasma membrane via its action on A_1 receptors (Kuroda et al., 1987; Ferrara and Cushman, 1999). While the role of adenosine in adipose tissue appears to be consensual, a lot of controversy exists regarding its action in skeletal muscle: Budohoski et al. (1984) showed increased insulin sensitivity in skeletal muscle in response to A_1 antagonist administration, while Thong and Graham (2002) described decreased insulin action in skeletal muscle after pharmacological inhibition of A_1 receptors. Also, Thong et al. (2007) showed that activation of the A_1 receptors increases insulin-stimulated glucose transport in rat soleus muscle. More recently, Faulhaber-Walter et al. (2011) demonstrated that A_1 knockout mice present decreased glucose tolerance with pronounced IR, suggesting that these receptors contribute significantly to whole-body glucose homeostasis and insulin sensitivity.

Alongside with the discussion on the role of A_1 receptors in glucose homeostasis, a role for A_{2B} receptors has also been proposed. Figler et al. (2011) described that A_{2B} antagonism increased insulin sensitivity and that these receptors control the release of inflammatory mediators, which can contribute to IR. Overall the role of adenosine and of its distinct receptors on glucose homeostasis needs to be clarified.

Caffeine is a non-selective antagonist of adenosine receptors and has also been implicated in skeletal muscle regulation of glucose metabolism. Caffeine blocks insulin-stimulated glucose uptake in rat skeletal muscle (Kolnes et al., 2010), stimulates insulin-independent muscle glucose transport (Jensen et al., 2007) and increases Glut4 mRNA content (Mukwevho et al., 2008) having metabolic properties similar to 5'-AMP activated protein kinase (AMPK). AMPK is a metabolic sensor that has been implicated in the control of muscle glucose homeostasis and lipid metabolism (Winder and Hardie, 1999). It has been shown that concentrations of caffeine above 3 mM increase insulin-independent glucose transport through an AMPK-mediated process (Egawa et al., 2009). Another key mediator in insulin sensitivity is nitric oxide (NO) (Guarino and Macedo, 2006). Steinberg et al. (1994) demonstrated that the inhibition of NO production induces IR and impairs glucose uptake by preventing insulin mediated vasodilatation in skeletal muscle. Also, there is a close relationship between adenosine and NO, since it is known that adenosine induces the release of NO via A_1, A_{2A} and A_{2B} receptors (Olanrewaju and Mustafa, 2000; Ray et al., 2002). Accordingly, caffeine modulates NO synthesis in several tissues (Corsetti et al., 2008; Ofluoglu et al., 2009).

The aim of the present study was to investigate the involvement of adenosine receptors on the effect of a single-dose caffeine administration on insulin sensitivity, disclosing the molecular mechanisms behind these effects in skeletal muscle. We demonstrated that acute caffeine administration decreases insulin sensitivity in a dose-dependent manner through the antagonism of A_1 and A_{2B} receptors. We also showed that this effect involves a decrease in insulin-stimulated glucose uptake in skeletal muscle via A_1 receptors. Additionally our results also demonstrated that NO is involved in acute caffeine induced-IR, being this effect mediated by adenosine receptors.

2. Materials and methods

2.1. Animals and experimental procedures

Experiments were performed in Wistar rats of both sexes (200–380 g), aged 9–12 weeks, obtained from the vivarium of the Faculty of Medical Sciences. The animals were kept under temperature and humidity control (21 ± 1 °C; 55 ± 10% humidity) with a 12 h light–12 h dark cycle. On the day before the experimental procedures, rats were fasted overnight and allowed free access to water. Afterwards, the animals were anesthetized with sodium pentobarbital (60 mg kg^{-1}, i.p.), since this anesthetic is the gold standard for metabolic studies as it does not alter plasma glucose, insulin or insulin sensitivity quantification (Guarino et al., 2013a,b). Catheters were placed in the femoral artery and vein of the animals for arterial blood pressure measurement and venous drug infusion. Animals were maintained in a heating pad to maintain body temperature throughout insulin sensitivity and blood pressure evaluation. For immunohistochemical analysis of adenosine receptors in the skeletal muscle, animals were anaesthetized with sodium pentobarbital (60 mg kg^{-1}, i.p.) and the protocol described in section 2.4 was followed. At the end of the experiments, rats were killed by an intracardiac overdose of pentobarbital, except when heart puncture was performed to collect blood. Principles of laboratory care were followed in accordance with the European Union Directive for Protection of Vertebrates Used for Experimental and Other Scientific Ends (2010/63/EU). Experimental protocols were approved by the ethics committee of the Faculty of Medical Sciences.

2.2. Measurement of insulin sensitivity

Insulin sensitivity was determined by the insulin tolerance test (ITT). The ITT provides an estimate of overall insulin sensitivity, correlating well with the 'gold standard' hyperinsulinemic–euglycemic clamp (Monzillo and Hamdy, 2003). It consists in the administration of an intravenous insulin bolus of 0.1 U kg^{-1} body weight in the tail vein, after an overnight fast, followed by the measurement of the decline in plasma glucose concentration over 15 min. The constant rate for glucose disappearance (K_{ITT}) was calculated using the formula $0.693/t_{1/2}$ (Monzillo and Hamdy, 2003; Guarino et al., 2013a,b; Ribeiro et al., 2013). Glucose half-time ($t_{1/2}$) was calculated from the slope of the least square analysis of plasma glucose concentrations during the linear decay phase. Blood samples were collected by tail tipping and glucose levels were measured with a glucometer (Precision Xtra Meter, Abbott Diabetes Care, Portugal) and test strips (Abbott Diabetes Care, Portugal).

To evaluate the effect of acute caffeine administration on insulin sensitivity a dose–response curve was performed. Fasting animals were submitted to an intravenous bolus of caffeine (0.001–5 μM), 15 min prior to the ITT. Dose–response curves for 8-cyclo-1,3-dipropylxanthine (DPCPX, A_1 antagonist, 0.0005–5 μM; Sigma, Madrid, Spain), 2-(2-Furanyl)-7-(2-phenylethyl)-7H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidin-5-amine (SCH58261, A_{2A} antagonist, 0.0005–0.5 μM; Tocris Bioscience, Abingdon, United Kingdom) and 8-(4-{[(4-cyanophenyl)carbamoylmethyl]-oxy}phenyl)-1,3-di(n-propyl)xanthine (MRS1754, A_{2B} antagonist 0.001–5 μM; Sigma, Madrid, Spain) were performed to investigate the adenosine receptors subtypes involved in the effect of acute caffeine administration on insulin sensitivity. DPCPX, SCH58261 and MRS1754 were dissolved in DMSO. To exclude possible effects of DMSO on insulin action, ITTs was also performed in rats where DMSO (1:1000, 1:10000, 1:50,000) was priory administered. DMSO dilu-