European Journal of Pharmacology 799 (2017) 154–159

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Full length article

Role of A_1 and A_{2A} adenosine receptor agonists in adipose tissue inflammation induced by obesity in mice

Caroline Candida DeOliveira, Cintia Rabelo e Paiva Caria, Erica Martins Ferreira Gotardo, Marcelo Lima Ribeiro, Alessandra Gambero*

Clinical Pharmacology and Gastroenterology Unit, São Francisco University Medical School, Av. São Francisco de Assis 218, 12916-900 Bragança Paulista, SP, Brazil

ARTICLE INFO

Keywords:
Obesity
Adipokine
Macrophage
A_{2A} adenosine receptor
CGS 21680

ABSTRACT

Adenosine receptors are expressed in adipose tissue and control physiological and pathological events such as lipolysis and inflammation. The aim of this study was to evaluate the activity of N^6-cyclopentyladenosine (CPA), a potent and selective A_1 adenosine receptor agonist; 2-*p*-(2-carboxyethyl)phenethylamino-5′-*N*-ethylcarboxyamidoadenosine hydrochloride (CGS-21680), an A_{2A} adenosine receptor agonist; and 5′-*N*-ethylcarboxamidoadenosine (NECA), a potent non-selective adenosine receptor agonist on adipose tissue inflammatory alterations induced by obesity in mice. Swiss mice were fed with a high-fat diet for 12 weeks and agonists were administered in the last two weeks. Body weight, adiposity and glucose homeostasis were evaluated. Inflammation in adipose tissue was assessed by evaluation of adipokine production and macrophage infiltration. Adenosine receptor signaling in adipose tissue was also evaluated. Mice that received CGS21680 presented an improvement in glucose homeostasis in association with systemically reduced inflammatory markers (TNF-α, PAI-1) and in the visceral adipose tissue (TNF-α, MCP-1, macrophage infiltration). Activation of p38 signaling was found in adipose tissue of this group of mice. NECA-treated mice presented some improvements in glucose homeostasis associated with an observed weight loss. Mice that received CPA presented only a reduction in the *ex vivo* basal lipolysis rate measured within visceral adipose tissue. In conclusion, administration of the A_{2A} receptor agonist to obese mice resulted in improvements in glucose homeostasis and adipose tissue inflammation, corroborating the idea that new therapeutics to treat obesity could emerge from these compounds.

1. Introduction

During conditions of cellular distress (inflammation, hypoxia, acute injury), extracellular adenosine is generated from hydrolysis of its precursor molecules, ATP, 5′-adenosine diphosphate (ADP) or 5′-adenosine monophosphate (AMP) (Fredholm, 2007; Fredholm et al., 2011). Extracellular adenosine can activate four subtypes of G protein-coupled receptors, A_1, A_{2A}, A_{2B} and A_3, and regulate several cellular processes (Fredholm, 2014). The A_1 adenosine receptors are highly expressed in adipose tissue and their activation results in lipolysis inhibition and modulation of insulin sensitivity (Faulhaber-Walter et al., 2011). Lipolysis itself can promote inflammation in adipose tissue (Mottillo et al., 2010), generating a vicious cycle of inflammation, insulin resistance and aberrant lipid flux in adipocytes. In this context, macrophages are an important source of proinflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)−6, during adipose tissue inflammation in obesity, and these cytokines contribute to insulin resistance in adipocytes through activation of c-Jun NH_2-terminal kinases (JNK)1 and JNK2. JNKs phosphorylate insulin receptor substrates and impair insulin signaling pathways (Hill et al., 2014) leading to increased lipolysis in adipocytes. Macrophages express all subtypes of adenosine receptors (Hasko and Cronstein, 2013). The anti-inflammatory effects of adenosine on macrophages are related to inhibition of pro-inflammatory cytokine, chemokine and nitric oxide release, and increases in IL-10 production, and these effects are mediated by A_{2A} and A_{2B} adenosine receptors (Hasko and Pacher, 2012). A_1 and A_3 adenosine receptors are expressed at lower levels than A_{2A} and A_{2B} adenosine receptors on the macrophage surface (Hasko and Pacher, 2012). Several studies have been published recently on diet-induced obesity using adenosine receptor knockout mice (Faulhaber-Walter et al., 2011; Figler et al., 2011; Johnston-Cox et al., 2012), and they provided valuable

* Corresponding author.
E-mail address: alessandra.gambero@usf.edu.br (A. Gambero).

http://dx.doi.org/10.1016/j.ejphar.2017.02.017
Received 12 October 2016; Received in revised form 9 February 2017; Accepted 10 February 2017
Available online 13 February 2017
0014-2999/ © 2017 Elsevier B.V. All rights reserved.

Table 1
Clinical and serum parameters of control, high-fat diet (HFD)- and adenosine receptor agonist-treated mice.

	Control	HFD	CPA		CGS		NECA	
			0.05 mg/kg	0.1 mg/kg	0.1 mg/kg	0.5 mg/kg	0.005 mg/kg	0.01 mg/kg
Body weight before treatment (g)	39.4 ± 1.0	57.6 ± 1.3	65.3 ± 3.2	62.5 ± 2.5	56.7 ± 1.9	57.2 ± 3.4	59.8 ± 3.9	53.7 ± 3.7
Body weight after treatment (g)	38.1 ± 1.0	58.0 ± 1.5	67.8 ± 2.3	64.6 ± 2.5	56.3 ± 1.9	56.5 ± 3.4	57.4 ± 3.3	48.5 ± 3.5
Variation of body weight (b.w.; %)	−3.4 ± 1.0	+0.6 ± 1.0	+4.2 ± 2.2	+3.6 ± 2.0	−0.6 ± 0.6	−1.1 ± 1.2	−3.8 ± 2.2	−9.8 ± 2.0[b]
Epididymal fat (% b.w.)	2.5 ± 0.1	3.6 ± 0.2[a]	3.6 ± 0.2	4.2 ± 0.3	3.7 ± 0.1	4.4 ± 0.6	4.1 ± 0.4	4.0 ± 0.3
Peri-renal fat (% b.w.)	0.4 ± 0.1	1.8 ± 0.4[a]	2.7 ± 0.5	1.6 ± 0.2	2.9 ± 0.2	2.6 ± 0.3	2.2 ± 0.1	0.3 ± 0.1[b]
Subcutaneous fat (% b.w.)	1.0 ± 0.1	3.8 ± 0.4[a]	3.9 ± 0.3	4.5 ± 0.3	4.2 ± 0.2	3.9 ± 0.5	3.5 ± 0.4	1.6 ± 0.4[b]
Glucose (mg/dl)	127 ± 5	215 ± 18[a]	219 ± 17	202 ± 24	144 ± 6[b]	155 ± 14[b]	159 ± 18[b]	152 ± 7[b]
Kitt	4.3 ± 0.3	1.9 ± 0.2[a]	1.8 ± 0.2	1.6 ± 0.4	2.4 ± 0.7	3.8 ± 0.4[b]	2.9 ± 0.4	2.5 ± 0.7
Insulin (ng/ml)	416 ± 201	1140+389[a]	1561 ± 143	1602 ± 523	1768 ± 334	673 ± 113[b]	1325 ± 332	1128 ± 245
Food intake (g/day)	4.3 ± 0.1	3.8 ± 0.1	3.4 ± 1.0	4.0 ± 1.0	4.1 ± 0.9	3.9 ± 0.5	5.0 ± 0.8	4.7 ± 1.0
TNF-α (pg/ml)	7.9 ± 0.4	9.6 ± 0.5[a]	9.6 ± 0.8	12.7 ± 3.6	8.4 ± 0.6	7.9 ± 0.3[b]	9.4 ± 0.9	8.7 ± 0.7
Leptin (pg/ml)	3379 ± 1408	9812 ± 1926[a]	6805 ± 1265	7451 ± 1333	8250 ± 894	10077 ± 1745	13265 ± 1515	10916 ± 1990
PAI-1 (ng/ml)	1440 ± 126	3513 ± 754[a]	4738 ± 530	4576 ± 1219	2455 ± 430	1988 ± 399[b]	3297 ± 778	3250 ± 609

[a] $P < 0.05$ when the HFD group was compared to the Control group.
[b] $P < 0.05$ when treated groups were compared to the HFD group (n=5).

Table 2
Adipokine production and basal lipolysis by adipose tissue of control, high-fat diet (HFD) and adenosine receptor agonists-treated mice.

	Control	HFD	CPA		CGS		NECA	
			0.05 mg/kg	0.1 mg/kg	0.1 mg/kg	0.5 mg/kg	0.005 mg/kg	0.01 mg/kg
TNF-α (pg/μg protein)	0.4 ± 0.1	0.8 ± 0.1[a]	0.7 ± 0.2	0.5 ± 0.1	0.3 ± 0.1[b]	0.4 ± 0.1[b]	0.6 ± 0.1	0.5 ± 0.0
IL-10 (ng/μg protein)	2.0 ± 0.7	2.0 ± 0.5	1.8 ± 1.1	2.7 ± 1.5	1.0 ± 0.3	1.3 ± 0. ± 3	2.8 ± 0.9	1.1 ± 0.6
Leptin (pg/μg protein)	0.1 ± 0.0	1.3 ± 0.1[a]	1.0 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.0 ± 0.3	1.0 ± 0.1	0.9 ± 0.3
Adiponectin (ng/μg protein)	8.2 ± 1.5	8.1 ± 1.0	8.1 ± 1.0	9.7 ± 0.4	8.3 ± 1.1	8.1 ± 0.9	11.6 ± 2.9	9.3 ± 1.2
MCP-1 (ng/μg protein)	1.0 ± 0.2	16.5 ± 2.3[a]	9.3 ± 3.6	6.1 ± 0.8[b]	7.1 ± 1.8[b]	6.4 ± 2.7[b]	6.4 ± 0.7[b]	8.6 ± 2.0
Glycerol (mg/dl)	0.4 ± 0.1	0.8 ± 0.1[a]	0.6 ± 0.1	0.6 ± 0.0[b]	0.7 ± 0.1	0.9 ± 0.1	0.7 ± 0.0	0.6 ± 0.1

[a] $P < 0.05$ when the HFD group was compared to the Control group.
[b] $P < 0.05$ when treated groups were compared to the HFD group (n=5).

contributions to the understanding of the adenosinergic system role in this context. In the present work, we intended to study the comparative ability of adenosine receptor agonists to control adipose tissue inflammation in mice fed a high-fat diet. Considering the well-established role of the A_1 adenosine receptor in lipolysis control and the A_{2A} adenosine receptor in inflammation control, we treated mice fed for 12 weeks with a high-fat diet with adenosine receptor agonists: N^6-cyclopentyladenosine (CPA), a potent and selective A_1 adenosine receptor agonist; 2-*p*-(2-carboxyethyl)phenethylamino-5′-*N*-ethylcarboxyamidoadenosine hydrochloride (CGS-21680), an A_{2A} adenosine receptor agonist; and 5′-*N*-ethylcarboxamidoadenosine (NECA), a potent non-selective adenosine receptor agonist.

2. Material and methods

2.1. Drugs

N6-cyclopentyladenosine (CPA) and 2-p-(2-carboxyethyl) phenethylamino-5′-N-ethylcarboxyamidoadenosine hydrochloride (CGS-21680) was purchase from Tocris, UK. 5′-N-ethylcarboxamidoadenosine (NECA) was purchase from Sigma-Aldrich, USA. Agonists were dissolved in DMSO 10% in saline solution. All agonists were administered by injecting a volume of 100 μl/65 g of body weight.

2.2. Animals

Six-week-old Swiss strain male mice, free of specific pathogens, were obtained from the Multidisciplinary Center for Biological Research (CEMIB; State University of Campinas, Campinas, SP, Brazil). Experiments were performed in accordance with the principles outlined by the National Council for the Control of Animal Experimentation (CONCEA, Brazil) and received approval from the Ethics Committee of São Francisco University, Bragança Paulista, SP, Brazil (Protocol 007.09.11). Animals were maintained on a 12:12 h artificial light–dark cycle and housed individually.

2.3. Diet-induced obesity and adenosine agonists treatment

After random selection, mice were introduced to control (15% energy from fat) or high-fat diets (HFD; 60% energy from fat) as previously described (DeOliveira et al., 2012). Body weights were assessed weekly. After 10 weeks, the HFD animals were randomly divided into seven groups. During the next two weeks, mice received intraperitoneal injections: CPA at doses of 0.05 and 0.1 mg/kg/day; CGS-21680 at doses of 0.1 and 0.5 mg/kg/day; NECA at doses of 0.005 and 0.01 mg/kg/day; vehicle (HFD group) at the same volume.

2.4. Blood glucose levels and insulin tolerance tests

In the last day of adenosine receptor agonist treatment, mice were deprived of food for 6 h and a blood drop was collected from their tails. Glucose was measured using the glucose oxidase method. Insulin (1.5 U/kg) was administered by an i.p. injection, and blood samples were collected for blood glucose determination at 0, 10, 15, 20 and 30 min. The rate constant for glucose disappearance during an insulin tolerance test (KITT) was calculated using the formula $0.693/t_{1/2}$. The glucose $t_{1/2}$ was calculated from the slope of the least square analysis of blood glucose concentrations during the linear decay phase.

2.5. Adipose tissue and blood collection

After 6 h of fasting, mice were anesthetized (i.p., 0.1 ml/30g of body

Download English Version:

https://daneshyari.com/en/article/5554729

Download Persian Version:

https://daneshyari.com/article/5554729

Daneshyari.com