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Adenosine receptors expression is elevated in leukocytes of gestational diabetes mellitus (GDM) subjects — a preliminary study

Podwyższona ekspresja receptorów adenozynowych w leukocytach pacjentek z cukrzycą ciążową — badanie wstępne

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Abstract

Introduction: Adenosine receptors (ARs), belonging to the G-protein-coupled receptors (GPCRs), are present in the majority of human cells and tissues. Depending on their biochemical and pharmacologic properties, four subtypes of ARs (*i.e.* A_1, A_{2A}, A_{2B}, and A_3) have been distinguished. Currently, these receptors are attractive molecular targets for pharmacological interventions in various diseases, including diabetes. The literature published to date has shown an altered expression of ARs in several types of cells under diabetic conditions. However, there has been no publication devoted to the investigation of ARs expression in leukocytes of subjects with gestational diabetes mellitus (GDM). Therefore, this study was aimed to determine the expression level of AR subtypes in leukocytes of GDM patients and its relationship to anthropometric and biochemical parameters.

Material and methods: Gene expression of four AR subtypes in leukocytes of both healthy (n = 34) and GDM (n = 67) subjects in the third trimester of pregnancy (from 24 to 33 weeks) was investigated. Multiple regression analyses were used to assess the association between the expression level of ARs and both anthropometric and biochemical parameters.

Results: Statistically significant ($p < 0.05$) higher levels of A_{2A} and A_{2B} mRNAs were observed in leukocytes of the GDM subjects compared to the control group. There was a positive correlation of A_{2B} mRNA level with glucose concentration at 120 min of oral glucose tolerance test (OGTT) ($r = 0.24$, $p = 0.041$).

Conclusions: Overexpression of A_{2B}AR in leukocytes of the GDM subjects and, additionally, the existence of a relationship between its elevated expression level in these cells and abnormal values of glucose concentration at 120 min of OGTT for GDM, suggest that this subtype might be involved in the pathogenesis of GDM. **(Pol J Endocrinol 2012; 63 (2): 110–114)**

Key words: *gestational diabetes mellitus, GDM, adenosine receptors, gene expression*

Streszczenie

Wstęp: Receptory adenozynowe (ARs) należą do błonowych receptorów sprzężonych z białkami G, które są obecne w większości ludzkich komórek i tkanek. Na podstawie ich biochemicznych i farmakologicznych właściwości wyróżniono cztery podtypy ARs (tj. A_1, A_{2A}, A_{2B}, A_3). Obecnie receptory te stanowią atrakcyjne molekularne cele dla farmakologicznych interwencji w różnych chorobach, w tym cukrzycy. W dostępnym piśmiennictwie istnieją dane wskazujące, że poziom ekspresji receptorów może ulegać zmianom w niektórych typach komórek podczas cukrzycy, natomiast nie ma publikacji dotyczących badania ekspresji ARs w leukocytach pacjentek z cukrzycą ciążową (GDM). Dlatego celem pracy było określenie poziomu ekspresji czterech podtypów ARs w leukocytach kobiet z GDN i ich korelacja z wybranymi parametrami antropometrycznymi i metabolicznymi.

Materiał i metody: Poziom ekspresji czterech podtypów ARs badano w leukocytach kobiet w ciąży, zarówno zdrowych (n = 34; grupa kontrolna), jak i ze zdiagnozowaną GDM (n = 67). Poziomy ekspresji korelowano z oznaczonymi parametrami antropometrycznymi i biochemicznymi.

Wyniki: W grupie GDM zaobserwowano istotny statystycznie ($p < 0,05$) wzrost poziomu mRNA receptorów A_{2A} and A_{2B} w porównaniu z grupą kontrolną. Zaznaczyła się również dodatnia korelacja między poziomem ekspresji receptora A_{2B} a stężeniem glukozy w 2. godzinie testu doustnego obciążenia 75 g glukozy ($r = 0,24$; $p = 0,041$).

Wnioski: Zarówno istotnie podwyższony poziom ekspresji receptora A_{2B} w leukocytach kobiet z GDM, jak i istnienie dodatniej korelacji między poziomem ekspresji receptora A_{2B} w tych komórkach a stężeniem glukozy wskazują na jego potencjalny udział w patogenezie GDM. **(Endokrynol Pol 2012; 63 (2): 110–114)**

Słowa kluczowe: *cukrzyca ciążowa, receptory adenozynowe, ekspresja genów*

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Introduction

To date, four adenosine receptor (AR) subtypes, termed A_1, A_{2A}, A_{2B}, and A_3, have been cloned from various species and characterised structurally, functionally and pharmacologically. It is now well established that all subtypes of ARs are transmembrane glycoproteins belonging to the G-protein-coupled receptors (GP--CRs) activated by adenosine [1–3]. Due to their wide distribution throughout the body, these receptors play an important role in many physiological and biochemical processes such as synaptic transmission, platelet aggregation, inflammation, glucose homeostasis and lipolysis [4, 5]. Moreover, ARs have been implicated in the pathogenesis of disorders such as cardiac and ischaemic diseases, asthma, chronic obstructive pulmonary disease (COPD), Parkinson's disease, retinopathy, cancer, obesity and diabetes [6–10]. With regard to the significance of ARs in type 2 diabetes mellitus (T2DM), considerable advances have been made towards explaining the role of the adipocyte A_1AR in the inhibition of lipolysis, and consequently an increase of insulin sensitivity [11, 12]. For this purpose, many studies using adenosine analogues both as agonists and antagonists have been carried out in cell-based systems and/or animal models [10, 13, 14]. Interestingly, recent studies have suggested a potential role for other AR subtypes in diabetes [15–17], but the underlying mechanisms remain poorly understood. The pathogenesis of GDM is still unclear [18]. The role of insulin resistance and insulin secretion disturbances are established, although new abnormalities have been recently found [19–21].

Since metabolic abnormalities such as chronic insulin resistance and pancreatic β-cell dysfunction characterise both T2DM and GDM [22], GDM gives the opportunity to investigate the early pathogenesis of T2DM [18, 23]. On the other hand, despite extensive studies into the role of ARs in T2DM, there is lack of knowledge concerning their role in GDM. Therefore, the aim of this study was to investigate the expression of the AR subtypes in leukocytes of GDM diagnosed women and (to correlate their expression level with anthropometric parameters *i.e.* body mass index [BMI] and body weight gain, as well as biochemical parameters, *i.e.* fasting: triglycerides [TGs], total cholesterol, high- and low-density lipoprotein HDL- and LDL-cholesterol), glycated haemoglobin (HbA_{1C}), and glucose at 0, 60, and 120 min of oral glucose tolerance test (OGTT).

Material and methods

Participants

The study group consisted of 101 white pregnant women. 67 of them had been diagnosed with GDM according to WHO criteria [24]. They were aged 29.6 ± 4.7 years (mean ± SD). There were also 34 healthy controls, aged 28.6 ± 4.2 years (mean ± SD), in the third trimester of pregnancy (24–33 weeks). All those participating in this study were recruited from the Polish Mothers' Memorial Hospital Research Institute in Lodz, Poland. Approval for this study was obtained from the Ethical Committee of the Medical University of Lodz, and informed consent was obtained from all participating subjects.

Biochemical measurements

Blood samples were drawn after a 12 h overnight fast. Serum TGs, and HDL- and LDL-cholesterol levels were determined by enzymatic colorimetric methods using triglyceride GPO-PAP and Total Cholesterol CHOD-PAP kits (Roche Diagnostics, Mannheim, Germany). OGTT was carried out with 75 g standardised glucose solution. HbA_{1c} was measured by immunoturbidimetry. Biochemical assays were carried out on a COBAS INTEGRA analyser (Roche).

Gene expression assay

Leukocytes, isolated from blood (10 mL) of healthy and GDM pregnant women, were suspended in TriReagent (Sigma-Aldrich, St Louis, MO, USA). Then total mRNA from these cells was extracted according to the manufacturer's instructions. The concentration of RNA and its purity were assessed by UV spectrophotometer LAMBDA 25 (PerkinElmer, UK) at UV_{260} and $UV_{260/280}$, respectively. The first cDNA strand was synthesised with the use of total RNA (4 μg), in the presence of a (dT) primer and RevertAid™ H Minus M-MuLV reverse transcriptase, according to the manufacturer's instructions (Fermentas, Lithuania). The expression level of ARs was analysed by a semi-quantitative PCR performed in a Thermocycler TPersonal 48 (Biometra, Germany). Gene expression was normalised to the housekeeping gene *GAPDH*. The primers for PCR were designed based on the GenBank database sequences and are listed in Table I. Each PCR reaction was run in duplicate in a solution containing 1 μL cDNA template, 1 μM primers (forward and reverse), 200 μM dNTPs, reaction buffer, and 0.6 U of *Taq* polymerase (Polgen, Poland), giving a final volume of 20 μL. The amplification products were analysed by 1.2% agarose gel electrophoresis and quantified by densitometry with the Gelix One 220 programme (Biostep GmbH, Germany). The ratio of AR/GAPDH was calculated for each sample.

Statistical analysis

The statistical analysis was performed with Statistica 8.0 software (StatSoft, Poland, licence no AXAP911E504325AR-K). Comparison between the results of the control and GDM groups was made with

Table I. *PCR primer sequences*
Tabela I. *Sekwencje starterów wykorzystywanych w PCR*

Gene	Primer sequence 5′ → 3′		Gene ID
	Forward	Reverse	
GAPDH	CTGCACCACCAACTGCTTAG	GTTGCTGTAGCCAAATTCGTTG	NM_002046
A_1AR	AATCTGAGTGCGGTGGAGC	CTCTTCTGGGAGATCCTCGTC	EF057066
A_{2A}AR	TCACTTTCTTCTGCCCCGACTGC	TGGCTAAGGAGCTCCACGTCTG	NM_000675
A_{2B}AR	GACAGTGCCACCAACAACTGCAC	CCAGCCTGACCATTCCCACTC	NM_000676
A_3AR	TCAGAGTACCACAGAAATGTCAC	CAGAATTCTTCTCAATGCTTGTGTC	NM_000677

Table II. *Anthropometric and clinical characteristics of control and GDM pregnant patients*
Tabela II. *Antropometryczna i kliniczna charakterystyka grupy kontrolnej i kobiet z GDM*

Parameter	Control patients (n = 34)	GDM patients (n = 67)	t test p value
Age (years)	28.6 ± 4.2	29.6 ± 4.7	0.307
BMI [kg/m^2]	24.0 ± 3.8	24.9 ± 5.7	0.389
Body weight gain [kg]	9.5 ± 5.7	9.7 ± 5.9	0.905
Total cholesterol [mg/dL]	254.4 ± 42.1	268.3 ± 42.5	0.270
HDL-cholesterol [mg/dL]	80.3 ± 14.3	69.2 ± 23.1	0.055
LDL-cholesterol [mg/dL]	131.7 ± 40.8	146.0 ± 42.4	0.248
TGs [mg/dL]	219.2 ± 55.4	276.3 ± 80.0	0.007*
HbA1c (%) Glucose [mg/dL]:	5.2 ± 0.4	5.4 ± 0.4	0.002*
at 0 min of OGTT	77.1 ± 8.0	90.1 ± 20.3	< 0.001*
at 60 mins of OGTT	145.0 ± 33.1	190.9 ± 31.1	< 0.001*
at 120 mins of OGTT	114.0 ± 21.9	165.0 ± 21.9	< 0.001*

Results are given as mean ± SD; *significant differences between the means of two groups of patients ($p < 0.05$)

the Student *t* test. Pearson correlation analysis was used to quantify relationships between the expression level of A_{2A} and A_{2B} ARs and the value of each anthropometric and biochemical parameter. Values were considered to be statistically significant when $p < 0.05$. The results are given as mean ± SD.

Results

Anthropometric and biochemical characteristics

Table II summarises the anthropometric and clinical characteristics of the study groups. No significant differences were found between the groups of patients either in anthropometric parameters (age, BMI, body weight gain during pregnancy) or in serum total cholesterol, HDL- and LDL-cholesterol concentrations. Significantly higher levels of serum glucose at 0, 60, and 120 min of OGTT as well as TGs and HbA_{1c} were found in GDM patients compared to healthy pregnant patients.

Expression levels of ARs mRNA

To evaluate the hypothesis of a possible role of ARs in the pathogenesis of GDM, we studied the gene expression of all four subtypes of ARs in blood leukocytes. We observed significantly higher levels of both A_{2A} and A_{2B} mRNA expression in leukocytes in the GDM group compared to the control group (A_{2A}AR: 0.84 ± 0.41 versus 0.55 ± 0.35, $p = 0.001$; A_{2B}AR: 0.94 ± 0.5 versus 0.64 ± 0.39, $p = 0.003$) as depicted in Figure 1A. In the cases of A_1 and A_3 ARs, there were no significant differences between the GDM group and the control group.

Next, we determined whether the expression levels of ARs were related to the anthropometric and biochemical parameters depicted in Table II. Our results revealed the lack of a significant relationship of leukocyte A_1, A_{2A} and A_3 mRNA expression with these parameters. A_{2B}AR mRNA level correlated significantly only with the glucose concentration at 120 min of OGTT ($r = 0.24$, $p = 0.041$) (Figure 1B) in the whole examined group.

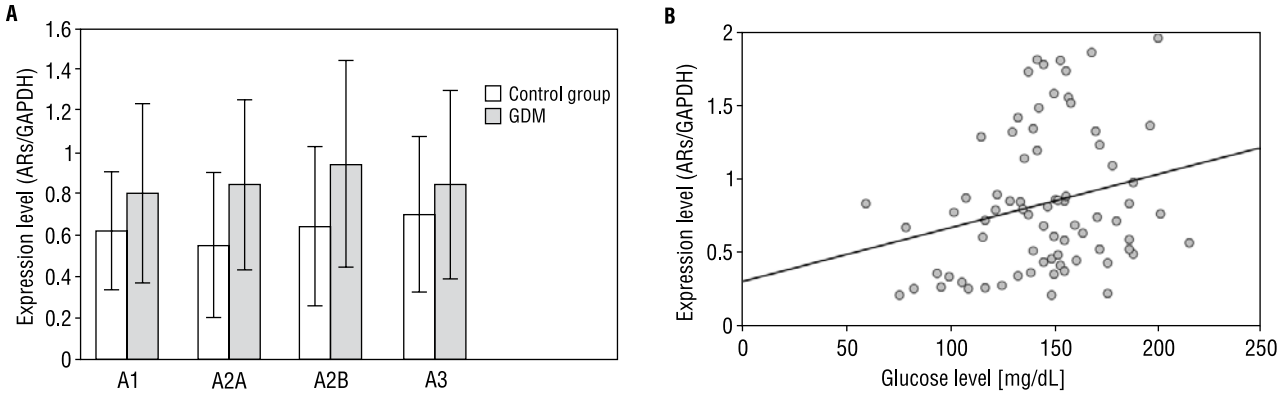

Figure 1. A. *Expression levels of four subtypes of ARs normalised to GAPDH mRNA in leukocytes of GDM patients. Control group (n = 34, white columns), GDM women (n = 67, grey columns);* **B.** *Positive correlation between A_{2B} expression level and a 2-h 75-g OGTT (r = 0.24, p = 0.041); *significant differences (p < 0.05)*

Rycina 1. A. *Poziomy ekspresji czterech podtypów receptorów adenozynowych normalizowane do GAPDH w leukocytach kobiet z GDM. Grupa kontrolna (n = 34, białe kolumny), kobiety z GDM (n = 67, szare kolumny);* **B.** *Pozytywna korelacja między poziomem ekspresji receptora A_{2B} a stężeniem glukozy w 2. godzinie testu OGTT (r = 0,24; p = 0,041); *różnice istotne statystycznie (p < 0,05)*

Discussion

GDM, a common metabolic disorder occurring during pregnancy, is defined as glucose intolerance that appears, or is first recognised, during pregnancy [25]. Metabolic abnormalities such as chronic insulin resistance and pancreatic β-cell dysfunction characterise both GDM and T2DM, and therefore GDM provides an opportunity to investigate the early pathogenesis of T2DM [22]. Although the mechanisms that account for insulin resistance in both GDM and T2DM are not completely understood, the role of ARs in T2DM, particularly A_1AR, has been identified. Indeed, A_1AR expression is increased in diabetes, and additionally, its activation by adenosine analogues (*i.e.* agonists) results in suppression of lipolysis associated with the reduction of circulating free fatty acid (FFA) levels [10, 13]. Recent studies have reported that the expression level of all ARs subtypes is changed in the liver, heart, and T lymphocytes of diabetic rats, suggesting a potential role in the pathogenesis of diabetes and its complications [15–17, 26, 27]. Moreover, the involvement of A_{2B}AR in diabetes was supported by a study with the use of A_{2B} receptor antagonist, 2-alkynyl-8-aryl-9-methyladenine, which suppressed glucose production from rat hepatocytes and had hypoglycaemic activity in a diabetic rat model [28].

Given that ARs represent attractive molecular targets for the development of drugs that might be used for the treatment of insulin resistance, metabolic syndrome, and T2DM, our particular interest was to determine whether the adenosine receptors mRNA levels are altered in the leukocytes of GDM women. It should be noted that although the distribution of ARs has been extensively studied, revealing the presence of all four types of these receptors on the various immune cell types [29], there is lack of knowledge about their expression levels in leukocytes of GDM patients. In this study, we show that leukocytes obtained from women with GDM are characterized by a significant increase ($p < 0.05$) in the A_{2A} and A_{2B} expression levels. Moreover, a positive correlation between the expression level of A_{2B} ARs in these cells and abnormal values of glucose concentration at 120 min of 75 g OGTT for GDM was found. This association is not surprising in the context of data showing that changes in the expression levels of ARs in diabetic T cells may result from hyperglycaemia and/or hypoinsulinaemia [26]. On the other hand, we cannot exclude the possibility that changes in the levels of hormones and/or oxidative stress during GDM might affect the A_{2A} AR and A_{2B}AR mRNA expression [30]. Therefore, the establishment of both the causes, and the functional consequences, of the altered expression of ARs should represent an exciting goal for further studies.

Conclusions

Our study demonstrates for the first time significantly higher expression levels of two subtypes of ARs, *i.e.* A_{2A} and A_{2B} ARs, in leukocytes of GDM subjects compared to healthy pregnant women; in addition, we demonstrated a positive correlation between the expression of A_{2B}AR and the glucose level at 120 min of OGTT. Therefore, A_{2B}AR seems to play an important role in glucose metabolism during GDM. However, whether these findings will be confirmed in larger studies with a broader representation among women with GDM remains to be determined.

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The study was approved by the Ethical Committee of the Medical University of Lodz, and the consent was obtained from each of the participating patients.

Conflict of interest

The authors state that there is no conflict of interest.

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