Effect of Caffeine Chronically Consumed During Pregnancy on Adenosine A₁ and A_{2A} Receptors Signaling in Both Maternal and Fetal Heart from Wistar Rats

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Background: Caffeine is the most widely consumed psychoactive substance in the world, even during pregnancy. Its stimulatory effects are mainly due to antagonism of adenosine actions by blocking adenosine A₁ and A_{2A} receptors. Previous studies have shown that caffeine can cross the placenta and therefore modulate these receptors not only in the fetal brain but also in the heart.

Methods: In the present work, the effect of caffeine chronically consumed during pregnancy on A₁ and A_{2A} receptors in Wistar rat heart, from both mothers and their fetuses, were studied using radioligand binding, Western-blotting, and adenylyl cyclase activity assays, as well as reverse transcription polymerase chain reaction.

Results: Caffeine did not significantly alter A₁R neither at protein nor at gene expression level in both the maternal and fetal heart. On the contrary, A_{2A}R significantly decreased in the maternal heart, although mRNA was not affected. Gi and Gs proteins were also preserved. Finally, A₁R-mediated inhibition of adenylyl cyclase activity did not change in the maternal heart, but A_{2A}R mediated stimulation of this enzymatic activity significantly decreased according to the detected loss of this receptor.

Conclusions: Opposite to the downregulation and desensitization of the A₁R/AC pathway previously reported in the brain, these results show that this pathway is not affected in rat heart after caffeine exposure during pregnancy. In addition, A_{2A}R is downregulated and desensitized in the maternal heart, suggesting a differential modulation of these receptor-mediated pathways by caffeine.

Introduction

CAFFEINE IS THE MAIN CONSUMED psychoactive substance around the world (more than 80% of the population) due to its presence in coffee, tea, and caffeinated soft drinks (including “energy” drinks).¹ The stimulant effect of caffeine at doses usually consumed by humans is due to the antagonism of adenosine. Adenosine is a nucleoside with actions on both the central and peripheral nervous systems being detected in the heart and endothelium, liver, nervous system, fat, and muscle. Different actions of adenosine are mediated through receptor binding, which have been classified into four types: A₁, A_{2A}, A_{2B}, and A₃ receptors. A₁ and A₃ receptors inhibit adenylyl cyclase (AC) activity through Gi/o protein and decrease cAMP level. On the contrary, A_{2A} and A_{2B} re-

ceptors activate AC activity, through Gs protein, causing a cAMP level increase.² As A₁ and A_{2A} receptors have a higher affinity with adenosine, these receptors seem to be the main ones blocked by caffeine.^{3,4} Caffeine is metabolized by the liver in theophylline and theobromine, which are also antagonists for adenosine receptors.⁵

Adenosine has been considered an important regulator within the cardiovascular system where it mediates important responses such as modulation of coronary flow, heart rate, and contraction.^{6,7} Adenosine has an antiadrenergic effect mediated through A₁ receptors⁸ and inhibits noradrenaline release from cardiac nerve terminals, being protective.^{9,10} The role of A_{2A} receptors in the heart should be expected to be more important at the high adenosine concentration (in the micromolar range) achieved after ischemic and hypoxic insults.⁶

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Cardioprotection has been associated with all subtypes of adenosine receptors,¹¹ although the role of each remains controversial.^{12,13}

Caffeine is widely consumed, even during pregnancy. However, there is insufficient evidence to confirm or refute the effectiveness of caffeine avoidance on birth weight or other pregnancy outcomes.¹⁴ This methylxanthine can reach fetal tissues because it can cross blood–brain¹⁵ and blood–placental¹⁶ barriers. Pregnant rats that have chronically consumed caffeine in their drinking water during all gestational period show a significant loss of A₁ receptors not only in the maternal whole brain but also in that of their fetuses.¹⁷ Moreover, the effect on the maternal brain depends on the region analyzed being increased in cingulate cortex (Cg2), Dentate Gyrus, and medial preoptic area, and decreased in the somatosensory cortex and striatum.¹⁸ The effect of caffeine during gestation was extended to the transduction pathway, including G-protein and adenylyl cyclase activity mediated by these receptors, causing a loss of system responsiveness only in the maternal brain, but downregulating G_{i1} protein in both the maternal and fetal brains.¹⁹ In addition, A₁R was also significantly decreased in the brain from mothers treated with caffeine only during lactation or throughout gestation and the lactation period. Interestingly, this receptor also diminished in the brain from 15-day-old neonates in a gender-dependent way, while A_{2A}R remained unaltered.²⁰

In the present work, we analyzed the effect of caffeine intake during gestation on adenosine receptors in the heart, where they are among the earliest expressed G protein coupled receptors.²¹

Materials and Methods

Materials

Cyclopentyl-1,3-dipropylxanthine,8-[dipropyl-2,3-H(N)] ([³H]DPCPX 120 Ci/mmol), 2-[p-(2-carboxyethyl)-phenethylamino]-5'-N-ethylcarboxamido adenosine ([³H]CGS21680 38,2 Ci/mmol), 5'-N-ethylcarboxamido [³H]adenosine ([³H]NECA 50 Ci/mmol) were from PerkinElmer (Madrid, Spain). Adenosine 3',5'-cyclic monophosphate ([³H]cAMP 28.4 Ci/mmol) was from New England Nuclear (NEN, Boston, MA). Calf intestine adenosine deaminase (ADA) and N⁶-cyclopentyladenosine (CPA) were from Sigma (Madrid, Spain). Anti-adenosine A₁ receptor was from Oncogene (Madrid, Spain). Anti- α G_{i1-2} and α G_{i3} antibodies were from Santa Cruz (Madrid, Spain). All other reagents were of analytical grade and obtained from commercial sources.

Animal treatment

Pregnant Wistar rats, kept on a 12 h/12 h light/dark cycle (lights on at 07.00 h) and with free access to food and drinking water, were treated with caffeine (1 g/L) in their drinking water from gestation day 2 (GD2) on-

wards during the entire gestation period. Control pregnant rats received drug-free tap water. At the end of this period, rats were sacrificed, and the fetuses were delivered surgically. Maternal and fetal hearts were then removed, frozen in liquid N₂, and stored at -80°C until experiments were performed. Daily water intake and rat weight were measured in all rats in the group. All experiments followed European Community and the Castilla-La Mancha University regulations for the care and use of laboratory animals.

Rat heart membranes isolation

Membranes were isolated as previously described.²² Hearts from pregnant rats were homogenized in 10 vol of ice-cold buffer (10 mM EDTA/10 mM HEPES/0.1 mM benzamidine, pH 7.4). Homogenate was centrifuged at 48,000 g for 10 min. The pellet was resuspended in 30 mL of buffer with 1 mM EDTA, centrifuged again, and washed two more times by resuspension/centrifugation. The final pellet was resuspended in 1 vol of the appropriate buffer (50 mM Tris-HCl + 10 mM MgCl₂) for assays. Membrane suspensions were stored at -80°C until use. Protein was determined by the Lowry method using bovine serum albumin as standard.

[³H]DPCPX, [³H]NECA, and [³H]CGS21680 binding assays to plasma membranes from rat heart

Binding assays to plasma membranes from mothers and fetuses were performed as described previously.¹⁷ Plasma membranes were incubated with 5 IU/mg adenosine deaminase (ADA) in 50 mM Tris, 2 mM MgCl₂, pH 7.4, for 30 min at 25°C in order to eliminate endogenous adenosine from membrane preparations. Then, plasma membranes (75 μ g of protein) were incubated with [³H]DPCPX for 2 h at 25°C. Saturation assays were carried out at different [³H]DPCPX concentrations (1–15 nM) using N⁶-cyclopentyladenosine (CPA) at a concentration 10⁴ times that of the radioligand in order to obtain nonspecific binding. Binding assays were stopped by rapid filtration through Whatman GF/B filters, preincubated overnight with 0.3% polyethylenimine, which were immediately washed three times with 4 mL ice-cold buffer. Filters were then transferred to vials and scintillation liquid was added to count the radioactivity. When A₂ receptors were assayed in 100 μ g of plasma membrane, [³H]NECA (50 nM) or [³H]CGS21680 (5–40 nM) were used and their specific binding displaced with 10 μ M CGS21680 or 10 μ M ZM241385 respectively.

Immunodetection of A₁R and α Gi proteins

Seventy to 100 μ g of protein were subjected to 7.5% polyacrilamide gel electrophoresis in the presence of SDS. Western blotting was performed as described earlier.¹⁹ Immunodetection was carried out by incubating the nitrocellulose membranes with specific polyclonal

antibody (anti- A_1 , anti- αG_{1-2} , and anti- αG_3). After washing, blots were incubated with horseradish peroxidase-coupled goat anti-rabbit or mouse IgG diluted 1:3,000. Antigen was visualized using the ECL chemiluminescent detection kit from Amersham, and specific bands were quantified by scanning densitometry in a GS-690 imaging densitometer from Bio-Rad (Madrid, Spain).

Reverse transcription polymerase chain reaction analysis

Total RNA was isolated by guanidium thiocyanate/phenol/chloroform extraction and reverse transcription polymerase chain reaction (RT-PCR) assays were performed as previously described.^{17,19} Primers used were: forward 5'-ATC CCA CTG GCC ATC CTT ATG-3' and reverse 5'-TGG CGA TGT AGA TCA GAA TGC-3' for A_1R ; 5'-CCA TGC TGG GCT GGA ACA-3' and 5'-GAA GGG GCA GTA ACA CGA ACG-3' for $A_{2A}R$; 5'-AAG GAC AGC GTG TGC AAG CCT GCT TCA AC-3' and 5'-AAT CTG TCA TTC CGT ACA AGG TAC TTA ACA-3' for αG_{11} , and 5'-GCA GGC TGC AAG GAG CAA CAG CGA-3' and 5'-CTC CTG GGT GCG CAC TGG GCC-3' for αGs . PCR products were analyzed by electrophoresis in 2% agarose gels and stained with ethidium bromide. The PCR product size expected for A_1 , A_{2A} , αG_{11} , and αGs were 630, 150, 1,080, and 770 bp respectively. In all cases, amplification of a fragment corresponding to the β -actin sequence was carried out in parallel using the same cDNA samples in order to correct possible variations in the amount of cDNA used. The primers used for β -actin were 5'-GGT ATG GAA TCC TGT CGC ATC CAT GAA A-3' and 5'-GTG TAA AAC GCA GCT CAG TAA CAG TCC G-3'. The size of the PCR product for the β -actin was 320 bp. Bands corresponding to PCR products were quantified using a GS-690 Imaging Densitometer from BioRad. In order to avoid working in saturation conditions, we optimized the number of cycles and cDNA quantity used.

Adenylate cyclase activity measurement

Adenylate cyclase (AC) activity was determined in heart plasma membranes as previously described.¹⁹ Assay was performed with 20 μ g of protein in a final volume of 0.25 mL of 50 mM Tris-HCl pH 7.4, 5 mM $MgCl_2$, 1 mM DTT, 1 mg/mL BSA, 1 mg/mL creatine kinase, 10 mM creatine phosphate, and 0.1 mM Ro 20-1724 (a specific phosphodiesterase inhibitor). Plasma membranes previously incubated with adenosine deaminase (0.2 IU/mg protein) at 37°C for 30 min in order to remove endogenous adenosine were incubated for 6 min at 30°C in the absence and in the presence of 5 μ M $GTP\gamma S$, 10 μ M forskolin, 100 μ M CHA, or 1 μ M CGS21680 followed by other incubation with 200 μ M ATP at 30°C for 10 min. Reaction was stopped by boiling samples and centrifugation at 12,000 g for 4 min. Supernatants (50 μ L) were used to determine cAMP accumulation.

Samples were incubated with 0.25 pmol of [3H]cAMP and 6.25 μ g cyclic AMP-dependent protein kinase in a final volume of 200 μ L of buffer assay (Tris-HCl 50 mM pH 7.4, EDTA 4 mM) during 2–18 h at 4°C. Standard samples were prepared in the same buffer in the range of concentration of 0–16 pmol. Reaction was stopped by the addition of 100 μ L of active charcoal solution containing BSA and centrifugation of the samples at 12,000 g for 4 min. Radioactivity was measured in a Beckman LS750 liquid scintillation counter.

Statistical and data analysis

Statistical analysis was performed using the Student's *t*-test. Differences between mean values were considered statistically significant at $p < 0.05$. Saturation (B_{max} , K_D) binding curves were analyzed, performing Scatchard and nonlinear regression analysis of binding data with GraphPad PRISM v3.02 (GraphPad Software, San Diego, CA).

Results

Caffeine intake was estimated from the loss of water from the drinking bottles. Daily water intake in the two groups of rats (control and caffeine treated: 83.2 ± 5.3 and 84.4 ± 5.5 mL/kg day respectively) was not significantly different during the period of study. This equates to a caffeine dose of approximately 84 mg/kg per day, which represents a high consumption model. The Human Equivalent Dose (HED) was calculated using the formula: $HED (mg/kg) = \text{rat dose} (mg/kg) \times \text{rat Km} / \text{human Km}$, where Km is a correction factor reflecting the

FIG. 1. Effect of caffeine on body weight gain. Body weight of each animal was measured every day in both caffeine ($n=8$) and control ($n=8$) groups as described in Materials and Methods. Data are mean $\pm$ standard error of the mean (SEM).

FIG. 2. Saturation curves of [³H]DPCPX specific binding to plasma membranes from maternal and fetal heart. Plasma membranes (75 μg of protein) from control and caffeine-treated pregnant rat heart of mothers (A) and fetuses (B) were incubated with different concentrations of [³H]DPCPX (1–15 nM), as described in Materials and Methods. Total receptors number (*B*_{max}) and receptor affinity (*K*_D) were determined by Scatchard analysis of saturation curves. Data are mean ± SEM of number of experiments in parentheses, each one performed in triplicate using different membrane isolations.

relationship between body weight and body surface area (equivalent to 6 and 37 for rat and human of 60 kg respectively).²³ This HED of 13 mg/kg day represents approximately 8 cups of coffee (100 mg/cup). Caffeine did not significantly alter the weight of mothers at the end of the gestational period (Fig. 1). There was no difference in the length of gestation between control and caffeine-treated animals. Macroscopic observation of the maternal and fetal hearts did not reveal malformations or significant variation in volume and weight between groups, in agreement with previously reported data.²⁴

Caffeine effect on adenosine A₁ receptors in maternal and fetal heart

A₁ receptors were quantified by radioligand binding assay using the selective antagonist [³H]DPCPX as radioligand, and CPA to obtain nonspecific binding. In the hearts from pregnant rats, no significant differences in total A₁R number or affinity were observed when caffeine was consumed (Fig. 2A). Similarly, no differences were observed in the fetal hearts (Fig. 2B).

In order to confirm these results, Western blotting assay was performed using specific anti-A₁ antibody.

FIG. 3. Immunodetection of A₁R in maternal and fetal heart. One hundred micrograms of plasma membranes from control and caffeine rat heart were subjected to SDS/PAGE, transferred electrophoretically to nitrocellulose membrane, and incubated with specific anti-A₁R antibody, as described in Materials and Methods. Data are mean ± SEM of at least three experiments performed using different membranes isolations from mothers (A) and fetuses (B). Inset shows representative experiments.

FIG. 4. A₁R expression detected by RT-PCR. RT-PCR assays were performed using specific oligonucleotides to A₁R, as described in Materials and Methods. The histograms show the ratio between amplification of A₁R and amplification of β-actin using the same RNA samples from mothers (**A**) or fetuses (**B**). Data are mean ± SEM of at least three experiments performed with different RNA isolations. Insets show representative experiments. RT-PCR, reverse transcription polymerase chain reaction.

FIG. 5. Specific [³H]CGS21680 and [³H]NECA binding to plasma membranes from maternal heart. Plasma membranes (100 μg of protein) from control and caffeine-treated pregnant rat heart of mothers were incubated with different concentrations of [³H]CGS21680 (5–40 nM) (**A**) or 50 nM [³H]NECA (**B**), as described in Materials and Methods. *B*_{max} and *K*_D values were determined by Scatchard analysis of saturation curves (**A**). Data are mean ± SEM of number of experiments in parenthesis, each one performed in triplicate using different membrane isolations. **p* < 0.05 significantly different from control values.

	<i>B</i> _{max} (fmol/mg prot)	<i>K</i> _D (nM)
<u>Mothers</u>		
CONTROL	23.0 ± 3.5 (5)	21.3 ± 1.3 (5)
CAFFEINE	10.5 ± 3.7 (4) *	7.4 ± 0.6 (4) *

FIG. 6. $A_{2A}R$ expression detected by RT-PCR. RT-PCR assays were performed using specific oligonucleotides to $A_{2A}R$, as described in Materials and Methods. The histograms show the ratio between amplification of $A_{2A}R$ and amplification of β -actin using the same RNA samples from mothers (A) or fetuses (B). Data are mean $\pm$ SEM of at least three experiments performed with different RNA isolations. Insets show representative experiments.

FIG. 7. $\alpha G_{i1,2}$ and αG_{i3} levels in maternal heart. Nitrocellulose membranes with controls and caffeine treated samples were probed with anti- $\alpha G_{i1,2}$ and anti- αG_{i3} antisera, as described in Materials and Methods. Data are mean $\pm$ SEM of at least three experiments performed using different membranes isolations. Inset shows corresponding bands to a representative experiment.

FIG. 8. αG_{i1} and αG_s expression detected by RT-PCR. RT-PCR assays were performed using specific oligonucleotides to αG_{i1} and αG_s as described in Materials and methods. The histograms show the ratio between amplification of αG_{i1} and αG_s and amplification of β -actin using the same RNA samples from mothers (A) or fetuses (B). Data are mean $\pm$ SEM of at least three experiments performed with different RNA isolations. Inset shows representative experiments.

No significant differences were observed in either maternal or fetal hearts (Fig. 3).

To verify whether caffeine consumption modifies expression of gene coding A_1R , RT-PCR assays were performed using total RNA isolated from maternal and fetal hearts, and no significant differences were observed between caffeine-treated and control groups in any case (Fig. 4).

Caffeine effect on adenosine A_{2A} receptors in the maternal heart

A_{2A} receptors were determined in maternal tissue by radioligand binding assay using different radiolabeled agonists. First, the radioligand [3H]NECA, nonselective A_2 agonist, was used, and no statistically significant decrease was observed (Fig. 5B). However, this decreased binding in the caffeine-treated group was significant when [3H]CGS21680, an A_{2A} selective agonist, was used as radioligand, with the total A_{2A} receptor number reduced in the caffeinated group to almost a half of that detected in control rats. Moreover, the K_D value also significantly decreased, suggesting a higher receptor affinity (Fig. 5A).

mRNA coding A_{2A} receptor was also assayed by RT-PCR, and no significant differences were observed between caffeine-treated and control mothers (Fig. 6A).

A_{2A} measurement by radioligand binding assay could not be performed in fetal samples because of the high amount of protein needed for the assay. However, RT-PCR assay revealed no differences in this fetal tissue between control and caffeine-treated groups (Fig. 6B).

Caffeine effect on G-proteins coupled to adenosine receptors

Alpha subunits of $G_{i1,2}$ and G_{i3} proteins were analyzed by Western blotting only in samples from the maternal heart. No significant differences were observed in any case (Fig. 7). Further, RT-PCR assays reflected no changes in the expression level of genes coding αG_{i1} and αG_s in both maternal and fetal heart samples (Fig. 8).

Caffeine effect on the transduction pathway mediated by A_1 and A_{2A} receptors

AC activity was determined in the plasma membranes from maternal and fetal hearts by quantitation

FIG. 9. Adenylyl cyclase activity in plasma membranes from maternal heart. AC activity was determined by quantification of cAMP levels by incubation of 30 μ g of plasma membranes previously treated with adenosine deaminase, with 10 μ M forskolin, forskolin + 5 μ M GTP γ S (panel A) or forskolin + 5 μ M GTP γ S + CPA (B) or CGS21680 (C). cAMP accumulation was measured as described in Materials and Methods. Data are mean $\pm$ SEM of at least five experiments performed in triplicate using different membrane preparations. * p < 0.05 and *** p < 0.001 significantly different from control values.

of cAMP level. Basal AC activity was significantly increased in caffeine-treated versus control samples in the maternal heart. Forskolin and Forskolin + GTP γ S were not significantly different between both groups of samples (Fig. 9A). In addition, the inhibitory effect on AC activity exhibited by CPA, selective A₁R agonist, was unaltered in the caffeine-treated group (Fig. 9B). On the other hand, in order to study whether the A_{2A}/AC pathway is affected by caffeine, AC activity was also

determined in the presence of CGS21680, selective A_{2A} agonist. A loss of the stimulatory ability of this ligand was observed, suggesting the desensitization of the transduction pathway mediated by A_{2A} receptors (Fig. 9C).

In the fetal heart, no significant differences were observed in basal, forskolin, and forskolin + GTP γ S conditions between control and caffeine-treated groups (Fig. 10A). The inhibitory response of AC exhibited

FIG. 10. Adenylyl cyclase activity in plasma membranes from fetal heart. AC activity was determined by quantification of cAMP levels by incubation of 20 μ g of plasma membranes previously treated with adenosine deaminase, with 10 μ M forskolin, forskolin + 5 μ M GTP γ S (A), or forskolin + 5 μ M GTP γ S + CPA (B), or CGS21680 (C). cAMP accumulation was measured as described in Materials and Methods. Data are mean $\pm$ SEM of at least five experiments performed in triplicate using different membrane preparations.

by CHA was also similar between groups, although there was less inhibition than in the maternal tissue (Fig. 10B). Contrary to what was observed in the maternal tissue, A_{2A} -mediated AC activity was similar in control and caffeine-treated groups, and a lower stimulation was detected (Fig. 10C).

Discussion

Modulation of adenosine receptors after caffeine exposure has been widely reported in the brain. However, less is known about this possible modulation in the heart.

Caffeine is rapidly absorbed from the gastrointestinal tract into the bloodstream and exerts central and peripheral actions, even crossing the placenta to arrive at fetal tissues.¹⁶ A growing body of research shows that coffee drinkers may be less likely to develop type 2 diabetes, stroke, depression, and neurodegenerative diseases, including Parkinson's and Alzheimer's.^{25–27} However, contradictory evidence in the literature about the effect of caffeine in the heart has been described. Thus, habitual moderate coffee intake has been associated with beneficial²⁸ or neutral²⁹ effects on cardiovascular health. Nevertheless, when chronically consumed, caffeine has been reported as a cardiovascular risk factor in women and men.^{30–32} Caffeine induces arrhythmias related to changes in cellular calcium homeostasis³³ and increases blood pressure,³⁴ increasing mortality from coronary heart disease and stroke.³⁵

Caffeine is widely consumed, even during pregnancy,^{36,37} and its reproductive and developmental risks have been evaluated.³⁸ Although caffeine has not been shown to be teratogenic in humans, even with regard to cardiovascular malformations,³⁹ consumption during pregnancy is associated with an increased risk of spontaneous abortions and reduced birth weight,^{40,41} particularly for those consuming 300 mg caffeine/day or more.⁴² In murine models, a single dose of caffeine during embryogenesis results in increased body fat and altered cardiac function in adulthood, indicating that caffeine exposure during early development can have long-lasting adverse effects,⁴³ and can even disrupt embryonic cardiac function.⁴⁴

Plasma caffeine levels in pregnant rats that were administered repeated doses of caffeine (80 mg/kg·per day) by oral intubation (gavage) and by administration in the drinking water on days 12–15 of gestation have been reported. The highest blood level of caffeine observed during a 24 h sampling period averaged 5.7 μ g/mL after drinking caffeinated water. The highest amount of caffeine observed in the blood averaged 63.1 μ g/mL 1 h after gavage.⁴⁵ The selected dose of caffeine (1 g/L in drinking water) has previously been shown in mice to attenuate depressor responses to adenosine significantly and to provide plasma caffeine concentrations of approximately 10 μ g/mL.⁴⁶ Therefore, in our experimental model, maternal caffeine plasma level would be sim-

ilar and not enough to inhibit PDEs. On the other hand, fetal plasma caffeine level has been reported to be one-half⁴⁷ to one-tenth²⁴ of the maternal level.

All adenosine receptors are expressed in the heart, and their therapeutic use has been studied.⁴⁸ The intact murine heart expresses high levels of Adora1 followed by Adora2a, with Adora2b and Adora3 more than two orders of magnitude lower.⁷ Adenosine in the heart has been shown to be cardioprotective, although some controversy remains with regard to acute myocardial infarction.^{12,13} Plasma adenosine concentrations are low during resting conditions but may be raised substantially in hypoxia, ischemia, and chronic heart failure.⁴⁹ As adenosine mediates coronary flow, heart rate, and contraction,^{6,7} its increase may attenuate the severity of chronic heart failure.⁵⁰ Adenosine exerts its cardiac electrophysiologic effects mainly through the activation of A_1 R.⁵¹ For instance, A_1 R activation by adenosine protects embryos against hypoxia in the heart,^{44,52–55} while embryos lacking cardiac A_1 R have decreased viability. Moreover, adenosine protects against vascular dysfunction via A_1 R while intrinsic A_{2A} activation apparently worsens contractile dysfunction.⁵⁶ Caffeine increases plasma adenosine levels,^{57,58} even when consumed during gestation.¹⁹ Therefore, caffeine exposure could represent a challenging status similar to hypoxic stimulus. The effects of caffeine are mainly due to blocking of adenosine actions primarily through A_1 and A_{2A} receptors, which modulate blood pressure and heart rate.⁵⁹ The absence of downregulation and desensitization of A_1 R in the heart, contrary to what has been observed previously in the brain, and the desensitization of A_{2A} R after caffeine intake during gestation suggest a physiologic resistance of this tissue against the possible noxious stimulus of this methylxanthine.

In our animal model, chronic maternal caffeine intake during gestation downregulates A_1 R in the whole brain, but does so differently depending on the brain area analyzed.¹⁸ This downregulation was also present in the brain from their fetuses.¹⁷ However, the A_1 R/AC pathway was only desensitized in the maternal brain.¹⁹ These effects are different to those obtained in their heart where A_1 R function is preserved, while A_{2A} R seems to be desensitized in the maternal heart, and both signaling pathways are preserved in the fetal heart. In agreement, there was no effect on A_1 R-mediated bradycardia induced by the specific receptor agonist R-PIA or on its mRNA expression in isolated hearts from male rats chronically treated with caffeine (0.6 g/L) for 2 weeks, although a lower heart rate was detected.⁶⁰ However, changes in resting heart rate and blood pressure concurrent with changes in A_1 R function have been previously reported in nonpregnant rats treated with a higher caffeine dose (2 g/L) for 2 weeks.⁶¹

Several factors could contribute to the increased basal cAMP levels detected in mothers after caffeine treatment. We have previously reported a significant reduction in the maternal heart of metabotropic glutamate

receptor level, including those negatively coupled to AC, which in turn decreases cAMP level.²² In addition to direct effects on the heart and vascular tone, adenosine can modulate the activity of the sympathetic nervous system. Thus, stimulation of presynaptic adenosine receptors can inhibit norepinephrine release from sympathetic nerve terminals.^{9,10,62} *In vivo* studies have suggested that A_{2A} and A₃ are responsible for this presynaptic effect.^{63,64} Moreover, a significant increase in the adrenaline plus noradrenaline content in the heart has been reported in rats ingesting high doses of caffeine.⁶⁵ This increase would elicit a higher activation of β -adrenergic receptors and, in turn, would also contribute to the detected higher basal cAMP level.

In summary, A₁R seems to be less sensitive to the effects of caffeine during gestation in the heart than in the brain, while A_{2A}R seems to be more vulnerable. This modulation could justify the pharmacological use of adenosine and related compounds, mainly through A₁R, in cardiovascular disorders.^{7,48,51,66,67} Taking into account these varied effects on both tissues, caffeinated beverages should be consumed with moderation during gestation.

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Author Disclosure Statement

No competing financial interests exist.

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