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Danger Signals From ATP and Adenosine in Pregnancy and Preeclampsia

Floor Spaans, Paul de Vos, Winston W. Bakker†, Harry van Goor, Marijke M. Faas

• Online Data Supplement

Preeclampsia is a multisystem pregnancy complication, which affects 2% to 8% of all pregnancies.¹ It is characterized by hypertension and proteinuria in the second half of pregnancy.² Although the complete pathophysiology is still unknown, it is thought to consist of 2 phases. The first phase is poor placentation, which may result in hypoxia of the placenta.^{3,4} The second phase is characterized by the release of proinflammatory factors from the hypoxic placenta, resulting in systemic inflammation and endothelial cell dysfunction. As a result, hypertension and proteinuria associated with potential damage to multiple organs may develop.^{3,4} Delivery of the placenta and the fetus is the only effective treatment option for the maternal symptoms.

High levels of ATP, which is now recognized as a danger signal, are found in preeclampsia.^{5,6} ATP is released by hypoxic and necrotic tissue, for instance by the hypoxic placenta.^{7,8} Release into the circulation causes activation of immune and endothelial cells,^{5,9} which in turn can also produce ATP, resulting in a cascade of activation.^{5,10} As a protective mechanism, ATP can be hydrolyzed into adenosine by various extracellular enzymes present in multiple cells including endothelial cells and placental trophoblast cells.^{11,12} Adenosine is also increased in preeclampsia and has opposite effects from ATP.¹³ Hence, the final effect of ATP and adenosine in preeclampsia depends on the balance between the 2 molecules. In the current review, we will discuss the role of ATP and adenosine in the pathogenesis of preeclampsia. We will first discuss the current knowledge on the biology of the 2 molecules in vascular function and the immune system, followed by an overview of how ATP and adenosine can play a role in pregnancy and preeclampsia.

ATP and Adenosine

Extracellularly, ATP serves as a danger-associated molecular pattern for the immune system.^{5,14} Danger-associated molecular patterns can initiate and prolong immune responses in an infection-free environment.¹⁵ ATP can be liberated after necrosis or necroptosis of cells.¹⁶ In addition, ATP release can be a regulated process. ATP is stored in secretory granules and can

be transported outside the cell via exocytosis.¹⁷ Also, various transmembrane channels (ie, connexins and pannexins) can release ATP into the extracellular space.¹⁸ Under physiological conditions, extracellular ATP concentrations vary between 400 and 700 nmol/L.¹⁹ During inflammation, hypoxia, or ischemia, ATP levels can increase 3-fold.^{5,20} This is for instance seen in diseases such as cystic fibrosis, chronic obstructive pulmonary disease, and preeclampsia.^{6,21,22}

To avoid ATP-induced pathological effects, cells can hydrolyze ATP into ADP and AMP by the enzymes ectonucleoside triphosphate diphosphohydrolase 1 (CD39) and alkaline phosphatase.¹¹ AMP can subsequently be broken down by 5'-ectonucleotidase (CD73) into adenosine and phosphate.¹¹ These enzymes are expressed in many tissues, including the placenta, and their activity and expression are changed in preeclampsia (Table S1 in the online-only Data Supplement).^{6,11} Adenosine generally counteracts ATP-induced effects.^{5,23} The final inflammatory effect of ATP depends on the balance between ATP and adenosine (Figure 1).

ATP and adenosine bind to purinergic receptors. Adenosine binds to the P1 receptors, ATP to the P2 receptors (P2X and P2Y receptors). Both subtypes of purinergic receptors have widespread tissue expression, including the placenta (see Tables S2 and S3).^{5,24,25}

ATP and Adenosine in Blood Pressure and Vascular Function

Extracellular ATP has been shown to regulate blood pressure in a dual, counteracting manner. Its effect seems to be correlated to the type of animal model used and the purinergic receptor involved.²⁶ In vivo it was shown that P2X₄ and P2X₁ receptor knockout mice display increased blood pressure because of a reduction in nitric oxide production.^{27,28} Knockdown of the P2X₇ receptor, however, resulted in a decrease in blood pressure.^{29,30} Because ATP is immediately hydrolyzed in vivo, it is unclear whether the above mentioned effects of ATP are related to ATP itself or to adenosine. More mechanistic insight into the role of ATP on vascular function is derived

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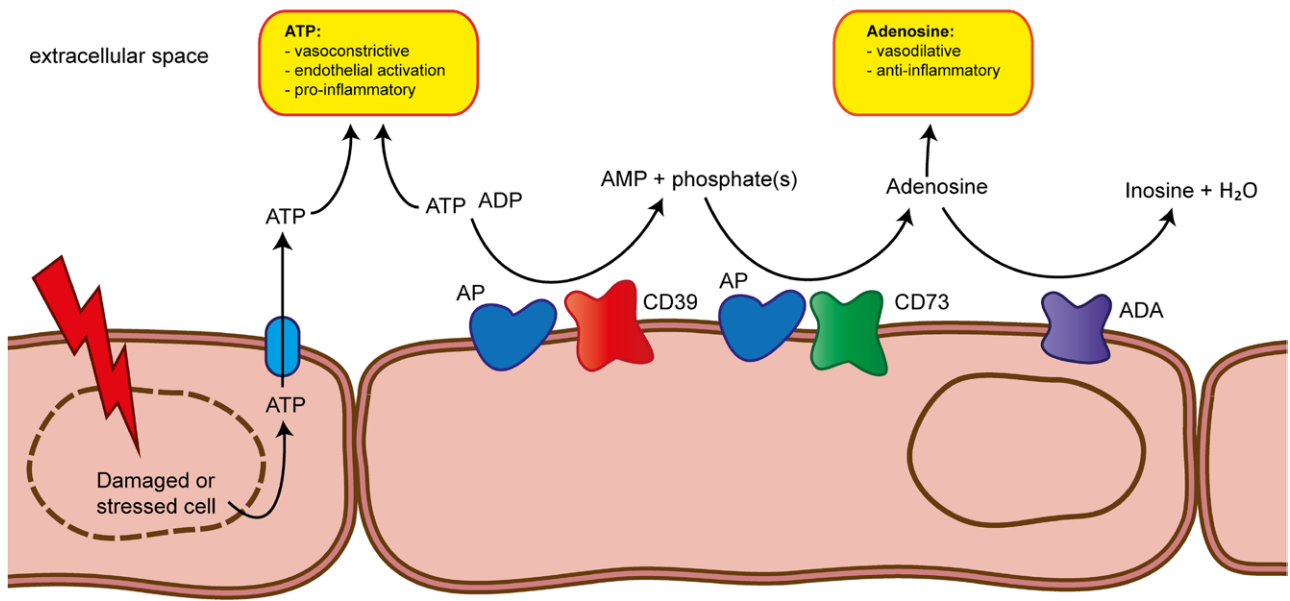


Figure 1. Schematic overview of ATP and adenosine metabolism in the circulation. Cellular stress and damage induce release of ATP into the extracellular space. ATP is hydrolyzed into ADP, AMP, and adenosine by CD39, CD73, and alkaline phosphatase (AP). Adenosine deaminase (ADA) reduces adenosine to inosine and water. Binding of ATP to P2 receptors induces vasoconstriction and inflammation, including endothelial activation and activation of inflammatory cells. Binding of adenosine to P1 receptors inhibits inflammation and induces vasodilation.

from several *in vitro* studies. ATP stimulation was shown to induce vasoconstriction of various arteries.^{31–34} These effects seem to be dose dependent, with a vasodilative response to lower ATP concentrations and a vasoconstrictory response to higher ATP concentrations.³⁵ The diverse vasoactive effects of high and low ATP may also be because of hydrolysis of ATP into adenosine, resulting in different ATP–adenosine ratios: low ratio after low ATP and a high ratio after high ATP. ATP stimulation of endothelial cells *in vitro* induced production of vasoactive substances, proinflammatory cytokines, chemokines, and adhesion molecules.^{9,36–39} ATP thus has vasoactive and proinflammatory effects.

The well-known vasodilatory effects of adenosine are mainly mediated by nitric oxide, via stimulation of A_{2B} receptors.^{40–42} However, adenosine can also have vasoconstrictive effects.⁴³ This was illustrated in A₁ receptor knockout mice, which demonstrated a decrease in blood pressure, whereas A₁ receptor agonists induced vasoconstriction and reduced glomerular blood flow.⁴⁴ Next to having a vasoactive effect, adenosine also acts as an anti-inflammatory molecule on endothelial cells.^{45,46}

Effects of ATP and Adenosine on the Immune Response

Almost all immune cells express purinergic receptors.⁵ ATP is involved in chemotaxis^{47,48} and can activate neutrophils^{48–50} and monocytes.^{47,51,52} Also here, at the level of the inflammatory system, adenosine seems to counteract the effects of ATP because adenosine suppresses neutrophil and monocyte–macrophage activation and recruitment *in vivo* and *in vitro*.^{53–56}

ATP also influences the specific immune response; *in vitro* stimulation of T cells with ATP induced T-cell activation and production of proinflammatory cytokines such as interleukin-2 and interferon- γ .⁵⁷ ATP stimulates the differentiation

of naïve T cells to proinflammatory T helper 17 (Th17) cells, whereas in the absence of ATP, the development of regulatory T cells is supported.^{58–60} CD39 is expressed by regulatory T cells and may be important for their regulatory and immunosuppressive action because, by hydrolyzing ATP and decreasing ATP concentration, it may induce differentiation of these regulatory T cells.⁶¹ Adenosine has opposite effects on T cells compared with ATP: *in vivo* and *in vitro* A_{2A} receptor stimulation promotes (1) long-term tolerance of T cells, (2) stimulates the induction of regulatory T cells, (3) reduced CD4+ Th1 and CD8+ Tc1 cell expansion to alloantigen, and (4) inhibits Th1- and Th2-cell development and effector function.^{62–64} Interestingly, stimulation of the A_{2B} receptor induced generation of Th17 cells.⁶⁵

The effects of extracellular ATP are activation of the inflammatory response and Th17 cells, whereas effects of adenosine are generally anti-inflammatory. Changes in the ATP–adenosine ratio toward one of the nucleosides may, therefore, determine either pro- or anti-inflammatory effects.

ATP and Adenosine During Normal Pregnancy

Adenosine, but not ATP, levels are increased in plasma from pregnant women.^{66,66} The elevated adenosine level may be explained by platelet activation (releasing ATP and ADP), increments in plasma activity of 5'-nucleotidases (CD73), or decreases in adenosine deaminase (ADA) activity during pregnancy.^{66,67} Also, ATP may be hydrolyzed faster during pregnancy because the ATP hydrolyzing enzymes CD39 and alkaline phosphatase are highly expressed in the placenta.^{12,68} These pregnancy adaptations suggest that extracellular ATP levels need to be regulated tightly during pregnancy.

The role of the increased adenosine in maintaining healthy pregnancy needs more investigation, but considering the

vasodilatory effect of adenosine, it may play a role in the hemodynamic changes in pregnancy.⁶⁶ During pregnancy, many maternal physiological adaptations are necessary to accommodate the developing fetus. For instance, blood volume and cardiac output rise by 50%, whereas blood pressure slightly decreases.^{69–72} Adenosine may also be important in angiogenesis of the fetus⁷³ and placenta because *in vitro* studies have shown that adenosine profoundly stimulates the production of proangiogenic factors such as vascular endothelial growth factor and membrane-bound fms-like tyrosine kinase-1 while inhibiting the antiangiogenic soluble fms-like tyrosine kinase-1.^{74,75} However, too much adenosine may be detrimental because mice deficient for ADA, which display increased adenosine levels, died during postimplantation period.⁷⁶ This suggests that adenosine regulation is essential for implantation and early development.⁷⁶

Although little is known about purinergic signaling in placental development or physiology, the finding that trophoblast cells carry almost all purinergic receptors, as well as CD39, alkaline phosphatase, and CD73, illustrates that purinergic signaling plays an important role (Tables S1–S3).^{12,25,77,78} Moreover, *in vitro* studies demonstrate that ATP stimulation increases intracellular Ca²⁺ levels in (primary) human and bovine trophoblast cells, indicating activation of these cells.^{79,80}

ATP and Adenosine in Preeclampsia

Both ATP and adenosine plasma levels are increased in preeclampsia compared with normal pregnant women. Unfortunately, ATP and adenosine have not been measured in the same patients, but a 2.5-fold increase in ATP⁶ and a 1.5-fold increase in adenosine^{13,81,82} suggest a rise in the plasma ATP–adenosine ratio of ≈ 1.5 -fold in women with preeclampsia compared with healthy pregnant women. This implies that the ATP–adenosine ratio in preeclampsia is shifted toward vasoconstriction and inflammation. The exact source of the rise in ATP and adenosine in preeclampsia is unknown, but it is possible that the hypoxic placenta, as well as activated immune and endothelial cells, releases increased amounts of ATP during preeclampsia.^{2,6} As outlined above, ATP may thus be one of the factors released by the hypoxic placenta in phase 2 of preeclampsia. Decreased hydrolysis of ATP may also occur in preeclampsia because CD39 expression was lower and CD73 expression higher in fascia and placentae from preeclamptic women compared with normal pregnant women.^{6,83} In patients with preeclampsia, compensatory mechanisms such as upregulation of alkaline phosphatase and increased ADA activity seem not to be effective in reducing the amount of extracellular ATP.^{84,85} The increased adenosine levels may be because of hydrolysis of ATP or increased platelet activation in preeclamptic women (Figure 1).¹³

Direct evidence for a pathophysiological role of ATP in preeclampsia arose from various animal experiments. Infusion of ATP into pregnant rats induced a preeclampsia-like syndrome including proteinuria and generalized inflammation.⁸⁶ Recent unpublished pilot studies in our laboratory showed that infusion of ATP (for 1 hour on day 14 of pregnancy) in pregnant rats induced a slight but significant increase in blood pressure until 48 hours after infusion. In addition, CD73 knockout

mice, which are likely to have elevated ATP levels, display preeclampsia-like symptoms, such as proteinuria, inflammation, endothelial dysfunction, and glomerular endotheliosis,^{87–89} whereas CD39 overexpression inhibited the induction of preeclampsia in mice.⁹⁰

Pathophysiological Role of Increased Plasma ATP and Adenosine in Preeclampsia

The mechanisms by which ATP induces its effects are not completely understood, but a direct effect of ATP on vascular function, as described above, is not unlikely.^{31–34} However, ATP may also increase blood pressure in preeclampsia indirectly, via activation of the inflammatory response (see below) or via inactivating hemopexin activity.^{91,92} Hemopexin is a free heme scavenger, which was recently shown to have serine protease activity.⁶ This protease activity increased during normal pregnancy, but not in preeclampsia, where its activity was inhibited by ATP.⁹¹ Because active hemopexin was shown to shed the angiotensin II receptor 1 from vascular cells, decreased hemopexin activity in preeclampsia, because of increased ATP, may result in increased angiotensin II receptor 1 expression and increased blood pressure.^{91,92} As far as the effect of ATP on the inflammatory response is concerned, ATP may be involved in activating inflammatory and endothelial cells, neutrophil and macrophage recruitment into arteries and the placental bed, induction of Th17 cells, and decreasing numbers of regulatory T cells in women with preeclampsia.^{58,59,65,93–98}

Increased adenosine levels in preeclampsia may also contribute to the pathogenesis of this disease. The finding that ADA-deficient mouse pups died in the postimplantation period suggests that high adenosine levels can inhibit placental development.⁷⁶ In addition, because adenosine stimulates nitric oxide production,⁴⁰ sustained higher adenosine levels could increase nitric oxide production, leading to the formation of peroxynitrite anion (ONOO⁻),⁹⁹ which contributes to endothelial dysfunction. Furthermore, increased A_{2B} receptors stimulation on T lymphocytes could increase Th17 formation,⁶⁵ whereas Th17 cells may contribute to the pathogenesis of preeclampsia.¹⁰⁰ Persistent high adenosine levels in preeclampsia may thus disturb endothelial function and contribute to immune activation in preeclampsia.

ATP and adenosine may have direct effects on the placenta. Because most of the P1 and P2 receptors are expressed in the placenta during pregnancy and preeclampsia, it seems likely that these sensory molecules have important roles in the development of and maintaining homeostasis in the placenta. Unfortunately, only a few studies are available addressing purinergic receptor expression in the placenta in preeclampsia. P1 and P2X₄ receptors were found to be increased in placental tissue from preeclamptic compared with normal pregnant women.^{24,101} Interestingly, under hypoxic conditions *in vitro*, placental explants from normal pregnancies showed increased expression of the A_{2A} receptor.²⁴ This may be a compensatory mechanism to increase the vasodilatory effect of adenosine. Such a hypoxia-induced increase in the A_{2A} receptor was not observed in the explants from preeclamptic pregnancies, suggesting that the preeclamptic placenta is unable to compensate in hypoxic conditions.²⁴

The question arises why ATP has a different effect in pregnancy compared with the nonpregnant situation because hypertension and proteinuria are not hallmarks of other diseases associated with increased ATP levels. Various suggestions can be put forward. First of all, the increased sensitivity to ATP during pregnancy may be because of the proinflammatory condition of pregnancy, which is characterized by activation of inflammatory cells.¹⁰² Pregnant individuals are more sensitive to proinflammatory stimuli: a proinflammatory stimulus in pregnant individuals induced a stronger and more persistent inflammatory response than in nonpregnant individuals.^{86,103} Therefore, it seems likely that ATP also induced a different inflammatory response in pregnant rats compared with nonpregnant rats. Second, not only the response to

proinflammatory stimuli has changed, it has also been shown that pregnant individuals are more sensitive to the products produced by inflammatory cells.¹⁰⁴ Therefore, even a minor activation of inflammatory cells, which does not affect nonpregnant individuals, may cause tissue damage in pregnant individuals. Finally, the presence of an additional vascular bed (the placenta) covered with purinergic receptors^{24,25,77} may explain why the response to ATP is different in pregnant compared with nonpregnant individuals.

Conclusions

Extracellular ATP and adenosine are in a delicate balance and tightly regulated by the enzymes CD39, alkaline phosphatase, CD73, and ADA to maintain normal pregnancy. Adenosine

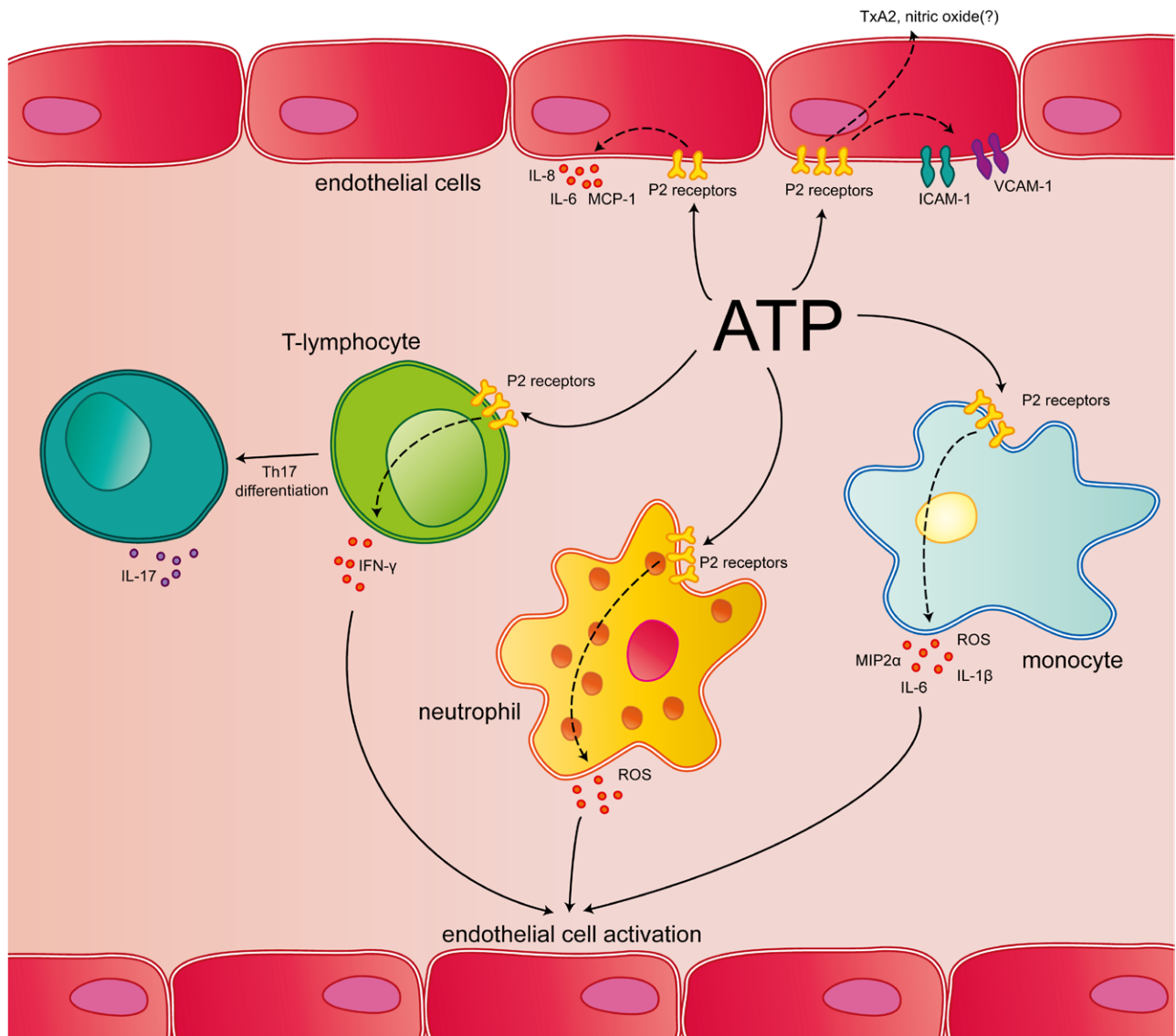


Figure 2. Postulated contribution of ATP to the pathogenesis of preeclampsia. High ATP levels (and ATP–adenosine ratio) activate endothelial cells, monocytes, neutrophils, and T lymphocytes. Stimulation of P2 receptors on endothelial cells induces expression of cytokines and chemokines, such as interleukin-6 (IL-6), IL-8, and monocyte chemoattractant protein 1 (MCP-1), and adhesion molecules such as vascular cell adhesion protein 1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), as well as vasoactive molecules. Monocyte and granulocyte activation by ATP leads to production of various proinflammatory substances, such as for instance reactive oxygen species (ROS), IL-1β, IL-6, and macrophage inflammatory protein 2α (MIP-2α). Binding of ATP to P2 receptors on T lymphocytes may induce interferon-γ (IFN-γ) production, as well as Th17 cell differentiation. Production of these factors by ATP may in turn activate endothelial cells and further stimulate the inflammatory response. NO indicates nitric oxide; and TxA2, thromboxane A2.

levels may be increased actively by platelet activation together with increased nucleotidase activity during normal pregnancy, and this may have beneficial effects on the vasculature, including vasodilation and avoiding hypertension. The ATP and adenosine balance is disturbed in preeclampsia, where both molecules are increased, but ATP to a higher extent, resulting in an increased ATP-adenosine ratio. This may induce hypertension, endothelial cell activation, and systemic inflammation (Figure 2). However, increased adenosine itself may also have negative effects on pregnancy. All signs point toward ATP as an important danger signal in preeclampsia. Modifying the ATP-adenosine ratio or interfering with purinergic receptors may provide opportunities for therapeutic intervention studies in preeclampsia in the future.

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None.

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ONLINE SUPPLEMENT

DANGER SIGNALS FROM ATP AND ADENOSINE IN PREGNANCY AND PREECLAMPSIA

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ATP/adenosine degrading enzymes and purinergic P1 and P2 receptors.

Expression of the enzymes involved in regulation of the ATP/adenosine balance (Table S1), P1 receptors (Table S2) and P2 receptors (Table S3), their relevant tissue expression and alterations of these enzymes and receptors that have been observed in preeclampsia.

Table S1. Enzymes involved in regulation of the ATP/adenosine ratio. n.d.= not determined.

Enzyme:	Function?	Expressed in placenta?	Location in placenta (reported)?	Activity in preeclampsia?	Other relevant tissue/cell expression
CD39	Hydrolysis of ATP and ADP into AMP	Yes	Cytotrophoblast cells, syncytiotrophoblast cells, endothelial cells	Decreased (fascia)	Neutrophils, monocytes/macrophages, T cells, endothelial cells, smooth muscle cells, kidney
CD73	Hydrolysis of AMP into adenosine	Yes	Trophoblast cells, endothelial cells, fibroblasts (?)	Increased (fascia), unchanged (placental bed)	T cells, endothelial cells, smooth muscle cells, kidney
Alkaline phosphatase (AP)	Hydrolysis of ATP, ADP and AMP into adenosine	Yes	Syncytiotrophoblast cells	Increased/decreased (plasma)	Neutrophils, monocytes/macrophages, T cells, endothelial cells, smooth muscle cells, kidney
Adenosine deaminase (ADA)	Breakdown of adenosine into inosine	Yes	Trophoblast cells	Increased (placenta)	Monocytes/macrophages, T cells, endothelial cells, smooth muscle cells, kidney

Table S2. P1 receptors and their expression in the placenta and other relevant tissues and in preeclampsia. n.d.= not determined.

P1 receptor:	Intracellular signaling	Purinergic ligand	Expressed in placenta?	Location in placenta?	Expression in preeclampsia?	Other relevant tissue/cell expression
A₁	↓cAMP	Adenosine/ Inosine, AMP	Yes	Trophoblast cells, endothelial cells, fibroblasts	Increased (placenta)	Neutrophils, monocytes/ macrophages, endothelial cells, smooth muscle cells, kidney Neutrophils, monocytes/ macrophages, T cells, endothelial cells, smooth muscle cells, kidney
A_{2A}	↑cAMP	Adenosine/ Inosine	Yes	Trophoblast cells, endothelial cells, fibroblasts	Increased (placenta)	Neutrophils, monocytes/ macrophages, T cells, endothelial cells, smooth muscle cells, kidney Neutrophils, monocytes/ macrophages, T cells, endothelial cells, smooth muscle cells, kidney
A_{2B}	↑cAMP	Adenosine	Yes	Trophoblast cells, endothelial cells	Increased (placenta)	Neutrophils, monocytes/ macrophages, T cells, endothelial cells, smooth muscle cells, kidney Neutrophils, monocytes/ macrophages, T cells, endothelial cells, smooth muscle cells, kidney
A₃	↓cAMP	Adenosine/ Inosine	Yes	Trophoblast cells, endothelial cells, fibroblasts	Increased (placenta)	Neutrophils, monocytes/ macrophages, T cells, endothelial cells, smooth muscle cells, kidney

Table S3. P2 receptors and their expression in the placenta and other relevant tissues and in preeclampsia. n.d.= not determined.

P2 receptor:	Intracellular signaling	Purinergic ligand	Expressed in placenta?	Location in placenta (reported)?	Expression in preeclampsia?	Other relevant tissue/cell expression
P2X₁	Ion channel	ATP	Yes (mRNA only)	Cytotrophoblast cells	n.d.	Neutrophils, monocytes/macrophages, T cells, endothelial cells, smooth muscle cells, kidney
P2X₂	Ion channel	ATP	Yes (mRNA only)	Cytotrophoblast cells	n.d.	Endothelial cells, smooth muscle cells, kidney
P2X₃	Ion channel	ATP	No	-	-	Endothelial cells, smooth muscle cells
P2X₄	Ion channel	ATP	Yes	Cytotrophoblast cells, syncytiotrophoblast cells, microvillous and basal membranes, fetal endothelial cells, Hofbauer cells(?)	Increased (placenta)	Neutrophils, monocytes/macrophages, T cells, endothelial cells, smooth muscle cells, kidney
P2X₅	Ion channel	ATP	No	-	-	Neutrophils, monocytes/macrophages, T cells, endothelial cells, smooth muscle cells
P2X₆	Ion channel	ATP	n.d.	-	n.d.	Monocytes/macrophages, endothelial cells, smooth muscle cells, kidney
P2X₇	Ion channel	ATP	Yes	Cytotrophoblast and syncytiotrophoblast cells	n.d.	Neutrophils, monocytes/macrophages, T cells,

P2Y₁	↑IP3	ADP (ATP)	Yes	Vasculature, Cytotrophoblast cells (mRNA)	n.d.	endothelial cells, smooth muscle cells, kidney Neutrophils, monocytes/ macrophages, T cells, endothelial cells, smooth muscle cells, kidney Neutrophils, monocytes/ macrophages, T cells, endothelial cells, smooth muscle cells, kidney Neutrophils, monocytes/ macrophages,
P2Y₂	↑IP3	UTP, ATP	Yes	Villous cytotrophoblast cells, syncytiotrophoblast cells	n.d.	T cells, endothelial cells, smooth muscle cells, kidney Neutrophils, monocytes/ macrophages,
P2Y₄	↑IP3	UTP (ATP in rodents)	Yes (mRNA only, no protein)	Cytotrophoblast cells	n.d.	T cells, endothelial cells, smooth muscle cells, kidney Neutrophils, monocytes/ macrophages,
P2Y₆	↑IP3	UDP	Yes	Villous cytotrophoblast cells and chorionic plate	n.d.	T cells, endothelial cells, smooth muscle cells, kidney Neutrophils, monocytes/ macrophages,
P2Y₁₁	↑IP3, ↑cAMP	ATP	Yes (mRNA)	Cytotrophoblast cells	n.d.	T cells, endothelial cells, smooth muscle cells, kidney
P2Y₁₂	↓cAMP	ADP	n.d.	-	n.d.	Monocytes/ macrophages,

P2Y₁₃	↓cAMP	ADP	n.d.	-	n.d.	T cells, endothelial cells, smooth muscle cells Monocytes/ macrophages,
P2Y₁₄	IP3	UDP, UDP- glucose, UDP- galactose	n.d.	-	n.d.	T cells, endothelial cells Monocytes/ macrophages,
