Danger signals from ATP and adenosine in pregnancy and preeclampsia

Floor Spaans¹ | Paul de Vos¹ | Winston W. Bakker²
| Harry van Goor² | Marijke M. Faas¹

University of Groningen and University Medical Center Groningen, Department of Pathology and Medical Biology, ¹Division of Medical Biology, ²Division of Pathology, Groningen, the Netherlands.

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Abstract
Preeclampsia is a multisystem complication in the second half of the pregnancy and characterized by hypertension and proteinuria. Although its specific pathophysiology is largely unknown, poor placentation, generalized inflammation and endothelial cell dysfunction are playing important roles. Recently, increased plasma levels of extracellular ATP have been found in women with preeclampsia. This increased ATP is considered to contribute to the development of the disease, since extracellular ATP has been shown to be a danger signal in many diseases. Extracellular ATP may increase blood pressure and activate endothelial cells and immune cells. In the circulation, ATP can be dephosphorylated to adenosine, which counteracts the effects of ATP. In the present review, we describe the importance of the delicate balance between extracellular ATP and adenosine in pregnancy and preeclampsia.
Introduction

Preeclampsia is a multisystem pregnancy complication, which affects 3-5% of all pregnancies [1]. It is characterized by hypertension and proteinuria in the second half of pregnancy [2]. Even though the complete pathophysiology is still unknown, it is thought to consist of two 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 pro-inflammatory 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 foetus is the only effective treatment option for the maternal symptoms.

High levels of adenosine triphosphate (ATP), which is now recognized as a danger signal, are found in preeclampsia [5,6]. ATP is released by hypoxic and necrotic tissue [7,8], for instance by the hypoxic placenta. 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 hydrolysed 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 two 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 two 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 (DAMP) for the immune system [5,14]. DAMPs 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 (i.e. connexins and pannexins) can release ATP into the extracellular space [18]. Under physiological conditions, extracellular ATP concentrations vary between 400-700 nM [19]. During inflammation, hypoxia or ischemia, ATP levels can increase three-fold [5,20]. This is for instance seen in diseases such as cystic fibrosis, COPD, and preeclampsia [6,21,22].

To avoid ATP-induced pathological effects, cells can hydrolyse ATP into ADP and AMP by the enzymes ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1 or 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 is changed in preeclampsia (Table 1) [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 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 KO mice display increased blood pressure, due to a reduction in nitric oxide (NO) production [27,28]. Knock down of the P2X7 receptor, however, resulted in a decrease in blood pressure [29,30]. Since ATP is immediately hydrolysed 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 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

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

Table 1. Enzymes involved in regulation of the ATP/adenosine ratio. n.d.= not determined.
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 hydrolysed 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.

An a vasodilatory response to low and a vasoconstrictory response to higher ATP concentrations [35]. The diverse vasoactive effects of high and low ATP may also be due to 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, pro-inflammatory cytokines, chemokines and adhesion molecules [9,36-39]. ATP thus has vasoactive and pro-inflammatory effects.

The well-known vasodilatory effects of adenosine are mainly mediated by NO, via stimulation of A2B receptors [40-42]. However, adenosine can also have vasoconstrictive effects [43]. This was illustrated in A1 receptor KO mice, which demonstrated a decrease in blood pressure, while 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 as adenosine suppresses neutrophil and monocyte/macrophage activation and recruitment in vivo and 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 pro-inflammatory cytokines such as IL-2 and IFN-γ [57]. ATP stimulates the differentiation of naïve T cells to pro-inflammatory Th17 cells while 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, since, by hydrolysing ATP and decreasing ATP concentration, it may induce differentiation of these regulatory T cells [61]. Adenosine has opposite effects on T cells compared to ATP: in vivo and in vitro A2A receptor stimulation promotes (i) long-term tolerance of T cells, (ii) stimulates the induction of regulatory T cells, (iii) reduced CD4+ Th1 and CD8+ Th1 cell expansion to alloantigen, and (iv) inhibits Th1- and Th2-cell development and effector function [62-64]. Interestingly, stimulation of the A2B receptor induced generation of Th17 cells [65].

The effects of extracellular ATP are activation of the inflammatory response and Th17 cells, while effects of adenosine are generally anti-inflammatory. Changes in the ATP-adenosine ratio towards one of the nucleosides may therefore determine either pro- or anti-inflammatory effects.
Table 3. P2 receptors and their expression in the placenta and other relevant tissues and in preeclampsia. n.d. = not determined.

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

Adenosine, but not ATP, levels are increased in plasma from pregnant women [6,66]. 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 ADA activity during pregnancy [66,67]. Also, ATP may be hydrolysed faster during pregnancy as the ATP hydrolysing enzymes CD39 and alkaline phosphatase are highly expressed in the placenta [12,68]. These pregnancy adaptations suggest that extracellular ATP levels need to be tightly regulated 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 foetus. For instance, blood volume and cardiac output rise by 50%, while blood pressure slightly decreases [69-72]. Adenosine may also be important in angiogenesis of the foetus [73] and placenta, since in vitro studies have shown that adenosine profoundly stimulates the production of pro-angiogenic factors such as VEGF and membrane-bound Flt-1, whilst inhibiting the anti-angiogenic sFlt-1 [74,75]. However, too much adenosine may be detrimental, as mice deficient for ADA, which display increased adenosine levels, died during post-implantation period [76]. This suggests that adenosine regulation is essential for implantation and early development [76].

Although little is known about purinergic signalling 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 signalling plays an important role (Table 1-3) [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 to normal pregnant women. Unfortunately, ATP and adenosine have not been measured in the same patients, but a 2.5 fold increase in ATP [6] and a 1.5 fold increase in adenosine [13,81,82] suggest a rise in the plasma ATP/adenosine ratio of about 1.5 fold in women with preeclampsia compared to healthy pregnant women. This implies that the ATP/adenosine ratio in preeclampsia is shifted towards 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 release 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 two of preeclampsia. Decreased hydrolysis of ATP may also occur in preeclampsia, since CD39 expression was lower and CD73 expression higher in fascia and placentae from preeclamptic women compared to normal pregnant
women [6,83]. In preeclamptic patients, compensatory mechanisms such as upregulation of alkaline phosphatase and increased ADA activity appear not to be effective in reducing the amount of extracellular ATP [84,85]. The increased adenosine levels may be due to 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 lab 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 KO mice, which are likely to have elevated ATP levels, display preeclampsia-like symptoms, such as proteinuria, inflammation, endothelial dysfunction and glomerular endotheliosis [87-89], while 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]. As active hemopexin was shown to shed the angiotensin II receptor 1 from vascular cells, decreased hemopexin activity in preeclampsia, due to 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 post implantation period suggests that high adenosine levels can inhibit placental development [76]. In addition, since adenosine stimulates NO production [40], sustained higher adenosine levels could increase NO production leading to the formation of peroxynitrite anion (ONOO-) [99], which contributes to endothelial dysfunction. Furthermore, increased A2B receptor stimulation on T lymphocytes could increase Th17 formation [65], while 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. As 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 to normal pregnant women [24,101]. Interestingly, under hypoxic conditions in vitro, placental explants from normal pregnancies showed increased expression of the A2A receptor [24]. This may be a compensatory mechanism to increase the vasodilatory effect of adenosine. Such a hypoxia induced increase in the A2A 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 as compared with the nonpregnant situation, since 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 due to the pro-inflammatory condition of pregnancy, which is characterized by activation of inflammatory cells [102]. Pregnant individuals are more sensitive to pro-inflammatory stimuli: a pro-inflammatory 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 as compared with nonpregnant rats. Secondly, not only the response to pro-inflammatory 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.

Conclusion
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 levels may be actively increased 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 towards 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.

References


[53] van der Hoeven D, Wan TC, Auchampach JA. Activation of the A(3) adenosine receptor suppresses superoxide production and chemotaxis of mouse bone marrow neutrophils. Mol Pharmacol 2008; 74:685-696.


