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Review

The resurgence of A2B adenosine receptor signaling ☆

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ABSTRACT

Since its discovery as a low-affinity adenosine receptor (AR), the A2B receptor (A2BAR), has proven enigmatic in its function. The previous discovery of the A2AAR, which shares many similarities with the A2BAR but demonstrates significantly greater affinity for its endogenous ligand, led to the original perception that the A2BAR was not of substantial physiologic relevance. In addition, lack of specific pharmacological agents targeting the A2BAR made its initial characterization challenging. However, the importance of this receptor was reconsidered when it was observed that the A2BAR is highly transcriptionally regulated by factors implicated in inflammatory hypoxia. Moreover, the notion that during ischemia or inflammation extracellular adenosine is dramatically elevated to levels sufficient for A2BAR activation, indicated that A2BAR signaling may be important to dampen inflammation particularly during tissue hypoxia. In addition, the recent advent of techniques for murine genetic manipulation along with development of pharmacological agents with enhanced A2BAR specificity has provided invaluable tools for focused studies on the explicit role of A2BAR signaling in different disease models. Currently, studies performed with combined genetic and pharmacological approaches have demonstrated that A2BAR signaling plays a tissue protective role in many models of acute diseases e.g. myocardial ischemia, or acute lung injury. These studies indicate that the A2BAR is expressed on a wide variety of cell types and exerts tissue/cell specific effects. This is an important consideration for future studies where tissue or cell type specific targeting of the A2BAR may be used as therapeutic approach. This article is part of a Special Issue entitled: "Adenosine Receptors".

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Since the initial demonstration that purines have prominent effects on cardiac function, the endogenous nucleoside adenosine has been considered a biologically active extracellular signaling molecule [1–9]. The dose–response effects observed with adenosine and synthetic adenosine analogues [10] in conjunction with the competitive antagonism by methylxanthines of adenosine's biological effects supported the idea of specific adenosine receptors [2,11,12]. Categorization of 'purinergic' receptors into adenosine activated 'P_1-purinoreceptors' and nucleotide activated 'P_2-purinoreceptors' was formally recognized by Burnstock in 1978 [13]. Based on their ability to inhibit or stimulate adenyl cyclase, adenosine receptors were subdivided into A_1 and A_2 subtypes, respectively [14,15]. The discovery of the existence of both high and low-affinity receptors that activated adenyl cyclase prompted further division of the A2 subtype [16,17]. The high-affinity receptor was designated the A2A adenosine receptor (A2AAR) and the low affinity, the A2B adenosine receptor (A2BAR) [18]. All adenosine receptors were originally cloned from a canine thyroid library [19] and the A2BAR was subsequently cloned from the rat hypothalamus [20,21], human hippocampus [22] and mouse mast cells [23]. Receptor homology between rat and mouse A2BAR is 96%, while the receptor shares 86% and 87% homology with the human receptor, respectively [21–23]. The human A2BAR is located on chromosome 17p11.2–p12 and contains a single intron that interrupts the coding region [24].

Elucidation of the functional role of the A2BAR was hampered by the lack of pharmacological agents with high specificity for the A2BAR [17,18,25–29]. This coupled with the fact that the A2BAR is often co-expressed with the pharmacologically similar A2AAR in many cell/tissue types led to the perception that A2BAR may not play an equally important biological role [27]. More recently, the notion that both adenosine levels [6,7,9,30–42] and A2BAR expression are dramatically induced in pathological conditions such as hypoxia or inflammation [7,30,33,34,43–54] gave new consideration to a role for the A2BAR under such conditions. In fact, several recent studies indicate a tissue protective role of the A2BAR in dampening hypoxia-induced inflammation and in promoting tissue adaptation to hypoxia [7,9,47,48,50,52,53,55–57].

1. Expression, regulation and signal transduction via the A2BAR

1.1. Cell and tissue expression pattern

Cloning of the A2BAR and the advent of RT-PCR facilitated specific determination of the expression pattern of the A2BAR [20]. Studies of isolated cultures and whole organs have demonstrated A2BAR expression in mast cells [23,58], macrophages [59], lymphocytes [50,60], neutrophils [9,61], dendritic cells [62,63], endothelial cells [7,9,48,50,64], myocardial cells [30,52,65], retinal epithelium [66], intestinal epithelium [47,54,67] and pulmonary epithelia [49,53], particularly on alveolar type II epithelial cells [68]. The original transcriptional analysis has been supported by the recent creation of a mouse model with targeted replacement of A2BAR exon 1 with a reporter gene allowing for specific elucidation of A2BAR expression [69]. In this mouse model, A2BAR is expressed in the brain, spleen, lung, colon, heart and kidney, where it is primarily localized to the vasculature [20,51,69]. The receptor is highly responsive to many biological processes and mediators, which induce its expression on a transcriptional [7,48] or post-translational level [49].

1.2. Transcriptional regulation of A2BAR in hypoxia

The low oxygen tension (hypoxia) present in cells and tissues under many disease states causes stabilization of the transcription factor hypoxia-inducible factor (HIF). Transcriptional induction by HIF occurs following binding of HIF to a consensus sequence known as the hypoxia responsive element (HRE) on its target gene. The functional HIF protein consists of a heterodimer of the α and β subunits which translocate to the nucleus following stimulation and activate specific target genes [70]. A recent study utilizing human A2BAR promoter constructs, site-directed mutagenesis and chromatin immunoprecipitation, demonstrated functional binding of HIF to a HRE site on the promoter resulting in increased A2BAR mRNA and protein [48]. Recent analysis has identified two other putative HRE binding sites on the human A2BAR promoter and one in the mouse promoter identical to the functionally confirmed site on the human promoter (Fig. 1) [48,71]. To date, HIF is the only functionally validated transcription factor for the A2BAR.

1.3. Novel A2BAR transcriptional regulators

The presence of putative binding sites for a number of transcription factors on the mouse and human A2BAR promoter has recently been described [71]. These include a putative site for NFκB on the human promoter — an important transcription factor in mediating inflammation, a process with which the A2BAR has been intimately linked [71]. Intriguingly, the human promoter contains a putative cAMP response element (CRE), which may implicate adenosine acting in an autoregulatory manner through upregulation of its second messenger cAMP [71]. Finally, analysis in this study suggests that there is an Nkx2.5 binding site in both the human and mouse A2BAR promoters [71]. This transcription factor is expressed in the developing heart where it regulates genes associated with cardiac development [72]. Furthermore, it has been implicated in protecting the heart from stress signals and maintaining a highly differentiated cardiac phenotype [73]. Since the A2BAR has been shown to play a protective role in cardiac disease [30,52,74] it is tempting to speculate that regulation of A2BAR expression by Nkx2.5 may be of physiological and/or pathophysiological relevance [30]. Overall, characterization of these novel transcription factor binding sites in the A2BAR promoter [71] points towards novel pathways by which A2BAR expression and/or function could be modulated.

1.4. Inflammation regulates A2BAR transcription

Multiple mediators associated with inflammation have been demonstrated to increase A2BAR expression. Bacterial lipopolysaccharide increases A2BAR mRNA expression in macrophages [75]. The

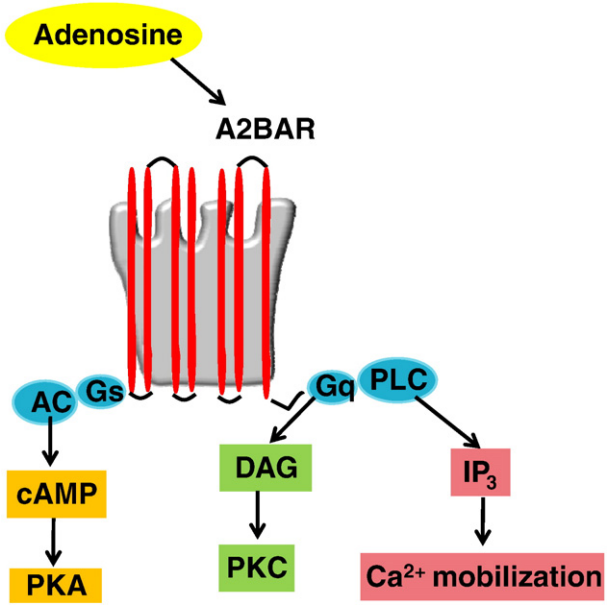

Fig. 1. A2BAR receptor signal transduction. Coupling of the A2BAR to Gs proteins results in cAMP upregulation and downstream PKA activation. Signaling through the Gq receptor leads to elevations in intracellular Ca^{2+} levels and an increase in PKC.

pro-inflammatory cytokine TNF-α increased A2BAR transcription in numerous cell types, both *in vitro* and *in vivo* [75–78]. It has been proposed that in vascular smooth muscle cells a TNF-α mediated increase in NAD (P) H oxidase enzymes is responsible for the upregulation of A2BAR expression [76]. In line with these studies, the pro-inflammatory cytokine, IL-1β, induced A2BAR transcription in endothelial cells [78]. Finally, IFN-γ was demonstrated to increase A2BAR mRNA expression in macrophages, which went hand in hand with increased cell surface expression of the receptor [59]. Interestingly, IFN-γ treatment of intestinal epithelial cells did not alter cell surface expression of the A2BAR [79]. This suggests that A2BAR receptor regulation by inflammatory mediators is likely to be cell type specific.

A recent study showed a novel mechanism for regulation of A2BAR transcript during inflammation [49]. A panel of inflammatory mediators, including PGE_2, IL-1β, IL-6 and IL-4 induces A2BAR mRNA in endothelial and pulmonary epithelial cells [49]. This was not associated with an increase in transcription but rather with stabilization of mRNA by these mediators pointing to a post-transcriptional regulation of A2BAR during inflammation [49]. In support of these observations a novel mechanism for regulation of A2BAR expression by TNF-α in intestinal epithelial cells has been proposed [80]. Potential binding sites for microRNA (miRNA) miR27b and miR128a in the 3 untranslated region of A2BAR have been identified [80]. Functional analysis demonstrated that both of these miRNAs can regulate A2BAR expression therefore pointing to a novel mechanism by which A2BAR expression can be regulated [80].

1.5. A2BAR sub-cellular localization

While initial analysis focused on cellular expression levels of the A2BAR, a number of studies have analyzed the cell surface expression of the A2BAR due to the importance of the sub-cellular localization of the receptor in its function [59,77,81]. Functional signaling through the receptor to its second messengers occurs when the receptor is at the cell surface [77,81]. In this regard adenosine itself has been demonstrated to regulate A2BAR cell surface expression [77,81]. In intestinal epithelial cells, A2BAR is located intracellularly at rest, and stimulation of basolateral or apical A2BAR receptors with adenosine can induce recruitment of the receptor to the apical cell surface [77,81]. Once at the plasma membrane, the receptor can interact with a larger complex of scaffolding proteins which may anchor and stabilize the protein at the cell membrane [81,82]. Furthermore, TNF-α potentiates the adenosine induced recruitment of the A2BAR to the plasma membrane in intestinal epithelial cells [77]. These studies demonstrate that adenosine signaling can be autoregulatory and that inflammatory mediators can potentiate this regulation in terms of A2BAR cell surface expression.

1.6. Ligand binding and signal transduction

The A2BAR has low affinity for its endogenous ligand, adenosine, with an EC50 of 24 μM [83]. However, it shows a preference for adenosine derivatives, with NECA (5′-*N*-Ethylcarboxamidoadenosine) being the most commonly utilized and potent A2BAR agonist, with an EC50 of approximately 2 μM [26–28]. The lack of specific A2BAR agonists has posed a particular problem in elucidating the specific function of the A2BAR when the A2AAR is also present [25,27,83]. Rational drug design using the structure of the receptor has been proposed as a mechanism for the development of more specific pharmacological agents [84–86]. The proposed structure of the A2BAR is typical of that of other G-protein coupled receptors (GPCR), with seven transmembrane (TM 1–7) domains composed of hydrophobic amino acids connected by three extracellular and three hydrophilic intracellular loops, bordered by an extracellular N-terminus and an intracellular C-terminus [21–23]. The highest homology between the A2BAR and the other adenosine receptors exists in the TM domains. Since the crystal structure of the first GPCR, bovine rhodopsin [87] was solved, many recent studies have worked on homology modeling using the known structure of this receptor to model the adenosine receptors [84–86]. The development of these models provided an exciting opportunity for rational drug design to target specific adenosine receptors. Combining these molecular models with known sequence data has prompted site-directed mutagenesis studies of the A2BAR to investigate specific receptor components involved in agonist and antagonist recognition. These studies have implicated amino acids in transmembrane region 3, 5, 6 and 7 in receptor recognition of agonists and antagonists [88,89]. However, to date mutagenesis studies of the A2BAR have revealed alterations in constitutive activity of the receptor but not in ligand binding activity [89]. The most recent study proposed a homology model of the A2BAR which was validated with known A2BAR agonists and antagonists [86]. This provides an important new tool for the development of more specific A2BAR pharmacological agents.

Presently, a novel compound (BAY-60-6583) has been developed with improved pharmacologic specificity for the A2BAR [30]. A more potent A2BAR antagonist is also available [90]. Both will be discussed in relation to therapeutic efficacy (3).

Upon ligand activation, the A2BAR can activate a number of signal transduction pathways to mediate its effects. Original studies in all cell types demonstrated that A2BAR couples to Gs to activate adenyl cyclase and increase intracellular cAMP levels (reviewed by Feoktistov and Biaggioni; Fig. 2) [91]. More recently it was shown that the A2BAR can couple to the Gq family of G-proteins to activate phospholipase C (PLC) and increase intracellular calcium (Fig. 2) [92,93]. It appears the G protein coupling of the receptor is cell type dependent and coupling to Gs results in an anti-inflammatory response while coupling to Gq could potentially results in a pro-inflammatory response [94]. Finally, the A2BAR is the only adenosine receptor subtype that can activate ERK1/2, JNK and p38 map kinase signaling [29,95–97]. There is an ongoing debate whether this is mediated through Gq or Gs coupling of the receptor. A2BAR specific signaling pathways will be further discussed in relation to specific disease pathologies (3).

2. A2BAR: physiologic and pathophysiologic function

Initial identification of the A2BAR as a low-affinity receptor for its endogenous ligand resulted in the A2BAR being considered less physiologically relevant that the high-affinity A2AAR [16,17,26]. The observation that receptor expression is dramatically enhanced in situations of low oxygen tension and inflammation (2.2, 2.4) coupled with the dramatic (100-fold) increase in extracellular adenosine levels under conditions of limited oxygen availability [98,99] point to a particular role for A2BAR during pathophysiological conditions. In addition, the alternate signaling pathways to which A2BAR can couple points to a potentially important role for A2BAR signaling distinct from that of the A2AAR (2.6, Fig. 2) [29,95–97].

2.1. A2BAR in vascular tone and vascular disease

The earliest observations on the physiological effects of adenosine point to a vaso-dilatory role for this nucleoside [3]. Initial studies

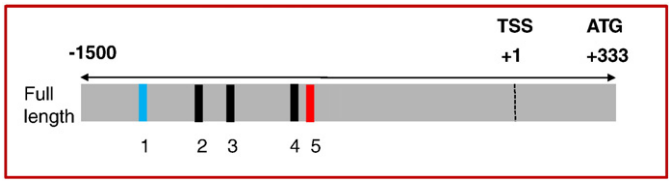

Fig. 2. Putative transcription factor binding sites in the human A2BAR promoter. 1 (TGAAGTG) is the proposed binding site for Nkx2.5, 2 (GCGTG), 3 (CACGC) and 4 (CACGT) are HIF-binding sites. 4 has been confirmed as an HRE bound by HIF-1 in hypoxia. 5 depicts the CRE binding site (CGTCA).

pointed to a primary role for A2AAR signaling, however the lack of effect of A2A specific agonists or other adenosine receptor antagonists in certain incidences revealed a contribution of the A2BAR receptor [100–102]. A2BAR expression has been detected in vascular endothelium and smooth muscle cells where it has been implicated in the regulation of vascular tone through receptor-mediated vaso-dilatory effects [100,101,103]. More specifically, A2BAR activation can inhibit vascular smooth muscle cell growth and induce endothelial cell growth [104–106]. Recent characterization of an A2BAR knockout reporter mouse (A2BAR-KO) supported the expression of the A2BAR in vascular cell types [69]. These mice do not display an obvious phenotype but at baseline they demonstrate a mild increase in pro-inflammatory cytokines and upregulation of adhesion molecules on the vasculature which is associated with IκB downregulation [69]. Furthermore, increased rolling of leukocytes and adhesion to vessel walls was observed in A2BAR-KO mice. These observations point to an important role for the A2BAR in attenuation of inflammation and maintenance of vascular tone.

Injury to the vasculature accompanied by accumulation of active inflammatory cells at the site of injury is important initiating events in vascular pathogenesis [69,107]. In a model of LPS acute inflammation and wire-induced vascular lesion, a significant increase in pro-inflammatory cytokines was observed in A2BAR-KO mice [69,107]. Bone marrow chimera experiments demonstrated that A2BAR expression on bone marrow derived cells contributed significantly to protection from vascular injury as measured by, cytokine levels, adhesion molecule expression and neointima formation [69,107]. This suggests that in certain vascular diseases A2BAR expression on hematopoietic cells can play a tissue protective role.

Novel evidence for a previously unappreciated protective role for the A2BAR in vascular disease was provided by a recent study using A2BAR-KO reporter mouse [108]. Historically, the inhibition of platelet aggregation by adenosine was attributed to signaling through the A2AAR [109–111]. However, it was recently shown that A2BAR expression is induced in murine platelets during response to stress and that A2BAR is expressed in human platelets [108,112]. A2BAR signaling enhanced cAMP which was responsible for inhibition of platelet aggregation [108]. Genetic loss of A2BAR resulted in increased platelet aggregation that was associated with changes in pro-aggregatory receptors in platelets. Taken together, this study suggests that the A2BAR plays a dual role in inhibition of platelet activation *in vivo*. The beneficial effect of A2BAR signaling in models of vascular injury and vascular cells points to a tissue protective role for A2BAR in diseases affecting the vasculature, such as atherosclerosis.

2.2. A2BAR in modulating inflammatory cell functions during hypoxia (leukomodulation)

Hypoxia is a well-documented inflammatory stimulus and results in tissue polymorphonuclear leukocyte (PMN) accumulation [5,56,113]. Likewise, increased tissue adenosine levels are commonly associated with hypoxia [5], and given the anti-inflammatory properties of adenosine, a study utilizing pharmacological and genetic approaches pursued the hypothesis that adenosine production via adenine nucleotide metabolism at the vascular surface triggers an endogenous anti-inflammatory response during hypoxia [9]. Initial *in vitro* studies indicated that endogenously generated adenosine, through activation of PMN A2BARs, functions as an antiadhesive signal for PMN binding to microvascular endothelia. Intravascular nucleotides released by inflammatory cells undergo phosphohydrolysis via hypoxia-induced CD39 ectoapyrase (CD39 converts adenosine triphosphate/adenosine diphosphate [ATP/ADP] to adenosine monophosphate [AMP]) and CD73 ecto-5′-nucleotidase (CD73 converts AMP to adenosine). Extensions of these *in vitro* findings using *cd39*- and *cd73*-null animals revealed that extracellular adenosine produced through adenine nucleotide metabolism during hypoxia is a potent anti-inflammatory signal for PMNs *in vivo* [9,35,113]. Moreover, a more recent study confirmed that *A2BAR*$^{-/-}$ mice experience increased inflammatory cell accumulation in hypoxic tissues, and that this phenotype can be resuscitated utilizing an A2BAR agonist (BAY-60-6583) [50]. Along the same lines, a third study implicated alternative mechanisms of adenosine receptor stimulation (via the neuronal guidance molecule netrin-1) in dampening inflammatory cell accumulation during hypoxia [57].Taken together, these findings indicate A2BAR signaling in dampening hypoxia-driven accumulation of inflammatory cells – particularly PMN – in inflamed or hypoxia tissues and suggest A2BAR signaling events in “leukomodulation”.

2.3. A2BAR in hypoxia, ischemia and ischemic pre-conditioning

Early evidence demonstrated that tissue hypoxia and ischemia significantly increase levels of extracellular adenosine [98,99,114–119]. Of the four adenosine receptors, the A2BAR is selectively upregulated during hypoxia [7,48]. Initial *in vitro* studies implicated hypoxia-dependent A2BAR expression in maintaining endothelial barrier function and an angiogenic phenotype [7,48]. The development of A2BAR deficient mice allowed for confirmation of this phenotype *in vivo*. A2BAR deficient mice or mice treated with an A2BAR specific antagonist experienced profound increases in vascular permeability upon hypoxia exposure [50]. In addition, A2BAR deficient mice experience increased hypoxia-induced neutrophil infiltration [50]. Importantly, a novel agonist with high specificity for the A2BAR (BAY-60-6583) was capable of attenuating hypoxia-induced permeability changes *in vitro* and *in vivo* [50]. Bone marrow chimeric studies pointed to a specific role for vascular A2B signaling in control of hypoxia-induced vascular leakage, while hematopoietic A2BAR was implicated in the regulation of hypoxia-associated neutrophil infiltration [50]. This indicates that A2BAR signaling on distinct cell types is protective in hypoxia-associated inflammation.

Ischemic pre-conditioning (IP) is an experimental approach in which exposure to repeated periods of vascular occlusion prior to an extended ischemic event provides dramatic protection from ischemic injury [120]. IP in both the heart and kidney is associated with dramatic increases in adenosine levels [30,121]. Given the tissue protective responses associated with adenosine it has been hypothesized that the protection provided by IP is related to modulation of adenosine levels [30,121]. To this end, the role of extracellular adenosine generation and the adenosine receptors in IP of the heart and kidney has recently been investigated [30,55,121]. IP in both organs specifically induces A2BAR expression, while mice with specific deletion of the A2BAR did not experience the protective effects of IP on the heart or lung [30,55,121]. Replacing IP with treatment using a novel A2BAR agonist (BAY-60-6583) prior to ischemia protected both organs from ischemia induced injury [30,121]. Furthermore, renal IP was associated with increased A2BAR expression on the renal vasculature [121]. Bone marrow chimeric studies revealed that A2BAR expression on the renal vasculature was responsible for protection of the kidney during IP, as measured by glomerular filtration and histology [121]. In line with these studies, expression of the A2BAR was specifically induced during intestinal ischemia/reperfusion (IR) injury, while specific A2BAR agonist and antagonist treatment revealed that targeting the A2BAR could be used therapeutically to attenuate intestinal inflammation, permeability and injury associated with gastrointestinal IR injury [47].

The availability of mice with genetic loss of the A2BAR allowed for development of the initial *in vitro* observations of a role for A2BAR during situations of low oxygen tension (hypoxia). *In vivo* studies using these mice in models of ischemia have demonstrated that A2BAR signaling on both hematopoietic and non-hematopoietic cell types play a protective role during the ischemic disease process. Importantly, the success of the novel A2BAR agonist, BAY-60-6583, in

attenuation of ischemic parameters provides therapeutic rationale for the use of A2BAR agonist therapy in ischemic disease.

The mechanism by which A2BAR signaling mediates tissue protection in models of ischemia and ischemia-associated inflammation has been investigated in an innovative study [122]. The authors demonstrated that adenosine is released by hypoxic preconditioned cells in a similar fashion to tissues exposed to ischemic pre-conditioning [122]. Adenosine signaling predominantly through the A2BAR set off a signaling cascade whereby the E3 ubiquitin ligase, cullin-1, was deneddylated rendering it incapable of ubiquitinating IκB-α and suppressing NFκB activity [122]. This is a novel and innovative finding which may point to the anti-inflammatory pathway responsible for the protective responses of A2BAR signaling during ischemic injury.

Over the last 10 years, there have been reports that the neuronal guidance molecule netrin-1 binds to the A2BAR and modulates signaling through the receptor [57,123]. Netrin-1 is induced at mucosal surfaces by hypoxia [57]. Intriguingly, netrin-1 signaling through the A2BAR on hematopoietic cells can inhibit hypoxia-induced inflammatory cell infiltration into mucosal organs [57]. Therefore, conventional A2BAR agonist signaling or alternative A2BAR signaling molecules could be a basis for future therapeutic strategies in ischemic disease.

2.4. A2BAR in acute kidney injury (AKI)

AKI is a devastating disease of the kidneys, that has previously been referred to as acute kidney renal failure. It is defined as a decrease in the glomerular filtration rate (GFR), occurring over a period of minutes to days. AKI is frequently caused by renal ischemia, and represents an important cause of morbidity and mortality of hospitalized patients [124–126]. A recent study of hospitalized patients revealed that only a mild increase in the serum creatinine level (0.3 mg/dl) is associated with a 70% greater risk of death than in persons without any increase [125,126]. Therapeutic approaches are very limited and the majority of interventional trials in AKI have failed in humans [127]. Therefore, additional therapeutic modalities to prevent AKI are urgently needed.

Several studies utilizing a model of isolated renal artery occlusion [128] to induce ischemic AKI have indicated a protective role of extracellular adenosine generation by dampening kidney inflammation and improving organ function [36,129]. Based on these findings, several studies pursued a kidney-protective role for extracellular adenosine signaling during ischemia, including studies on the A1AR [1,2,130], or the A2AAR [131,132]. A recent study by Grenz et al. attempted to compare adaptive responses elicited by IP treatment of the kidneys in all four adenosine receptor deficient mice. These studies determined a selective role for the A2BAR for IP-dependent kidney protection [51]. Moreover the authors could re-capitulate an *A2BAR*$^{-/-}$ phenotype by pre-treatment with an A2BAR antagonist (PSB1115). Finally, studies utilizing an A2BAR agonist (BAY-60-6583) were associated with robust protection from AKI in wild-type, and essentially no effect in *A2BAR*$^{-/-}$ mice. Studies utilizing a transgenic A2BAR reporter mouse [69,107], in conjunction with functional experiments in A2BAR bone marrow chimera indicate that A2BAR-dependent kidney protection involves vascular A2BAR signaling events [51]. However, these studies will require confirmation on a tissue-specific level, e.g. by utilizing the Cre-lox system to confirm that adenosine-A2BAR-dependent kidney protection from AKI involves vascular A2BARs.

2.5. A2BAR in acute lung injury

Acute lung injury (ALI) is a syndrome characterized by acute hypoxemic respiratory failure in the setting of non-cardiogenic pulmonary edema [133]. The pathogenesis of ALI is characterized by the influx of a protein-rich edema fluid into the interstitial and intra-alveolar spaces as a consequence of increased permeability of the capillary-alveolar barrier [133]. Molecular aspects of how capillary-alveolar leakage is caused and maintained during ALI remain unknown, and studies linking its mechanisms with mechanical ventilation or mechanical stretch are areas of intense investigation [117,134–136]. Initial studies using a murine model of ventilator induced lung injury (VILI) to model ALI in the setting of mechanical ventilation and stretch demonstrated elevated levels of extracellular adenosine in the lung [31]. Similarly, an *in vitro* model of cell stretch demonstrated the release of adenosine from lung epithelial cells cultured under stretch conditions [31]. Mice deficient in the enzymes required for the generation of extracellular adenosine were more susceptible to capillary-alveolar leakage and lung inflammation in the VILI model [31]. This suggested that extracelluar adenosine and adenosine receptor signaling pathways are protective in ALI. The availability of mice with genetic deletion of the individual adenosine receptors allowed for dissection of the receptor responsible for the protective effects of adenosine in ALI [136]. Specific enhancement of albumin leakage and decrease in survival was observed in A2BAR mice subjected to VILI [136]. This initial finding demonstrated a specific role for the A2BAR in acute lung injury. Previous study had demonstrated attenuation of pulmonary inflammation during hypoxia by A2BAR signaling [122]. Lung inflammation was assessed by neutrophil accumulation and cytokine levels in the lung as a result of VILI [136]. Mice deficient in the A2BAR demonstrated dramatic enhancement in pulmonary neutrophil numbers and pro-inflammatory cytokines (TNF-α, IL-6 and KC) following VILI [136]. Interestingly, levels of anti-inflammatory cytokine IL-10 were significantly decreased in the A2BAR deficient mice [136]. In a support of an anti-inflammatory role for A2BAR signaling in VILI, pulmonary A2BAR expression was induced during VILI and was associated with cAMP and PKA activation [136]. The specific role of the A2BAR in inducing pulmonary cAMP was confirmed using A2AAR deficient mice [136]. Studies performed with two distinct A2BAR antagonists confirmed the observations in A2BAR deficient mice [31,136]. Finally, a novel A2BAR agonist (BAY-60-6583) improved survival, albumin leakage and attenuated pulmonary inflammation in both VILI and endotoxin induced ALI [136]. Bone marrow chimeric studies revealed a dual role for A2BAR during VILI with pulmonary A2BAR attenuating lung edema and albumin leakage, while both pulmonary and hematopoietic A2BAR contributed to attenuation of inflammation during VILI [136]. The protective effect of A2BAR signaling on capillary-alveolar leakage is consistent with previous observations of A2BAR-dependent maintenance of barrier function [50,69,107]. In parallel with the protective effect of A2BAR on inflammatory parameters in VILI, hematopoietic A2BAR was demonstrated to be essential for hypoxia-associated neutrophil accumulation [50]. In a complimentary study, mice were exposed to inhaled LPS to mimic sepsis induced acute lung injury [49]. A reproducible protective effect of A2BAR signaling in this model provided further confirmation for a tissue protective role for A2BAR in acute lung injury [49]. These studies suggest that targeting of the A2BAR during ALI would be of therapeutic benefit, both in the setting of improving pulmonary barrier function and inflammation associated mechanical or sepsis induced lung injury.

2.6. Asthma and chronic lung disease

In contrast to the beneficial effects of adenosine-A2BAR signaling in acute conditions affecting the lung, the role of elevated adenosine levels in chronic lung pathologies, including asthma and chronic obstructive pulmonary disease (COPD) is somewhat more controversial [90,137–140]. Original studies identified elevated levels of adenosine present in the lungs of asthmatics which correlate with the degree of the inflammatory insult [141,142]. Importantly, adenosine induces acute bronchoconstriction in asthmatics or COPD sufferers, but not in normal individuals, pointing to an underlying

alteration in adenosine signaling in these patients [143,144]. Studies with mice deficient in the enzyme that deaminates adenosine to inosine – adenosine deaminase (ADA) – have elevated levels of adenosine present in the lung [145]. The elevated levels of adenosine in these mice correlate with pulmonary inflammation and airway remodeling symptoms [145]. Studies in ADA-deficient mice [145,146], mice with partial ADA deficiency [147,148] and mice undergoing ADA replacement therapy [149] demonstrate adenosine-dependent mast-cell degranulation, increase in number of lung macrophages and eosinophils, mucous metaplasia, airway enlargement and hyper-responsiveness. Interestingly, mice that are incapable of extracellular adenosine generation experience enhanced lung inflammation and fibrosis in a model of lung fibrosis [138]. The paradoxical effects of adenosine in these studies points to potentially differential effects of adenosine during chronic lung disease which may be due to the involvement of different adenosine receptor signaling pathways on distinct cell types.

Original studies using an adenosine uptake inhibitor implicated cell surface receptors in adenosine-mediated bronchoconstriction in asthmatics [150]. Further evidence for specific adenosine receptor involvement was illustrated by the efficacy of adenosine receptor antagonists in adenosine-mediated bronchonstriction [151,152]. Degranulation of mast cells and subsequent mediator release is an important component of the bronchoconstriction observed in asthma [153]. Importantly, investigation of adenosine receptor subtypes on mast cells implicate A2BAR signaling in degranulation and mediator release [23,58]. A2BAR induced mediator release from mast cells is facilitated by the Gq pathway and can be enhanced by Gs signaling [95,154]. In an extension of these studies, A2BAR signaling has been implicated in increasing cytokine release from other cell types implicated in asthma, including airway epithelial cells [155], bronchial smooth muscle cells [156], macrophages [157] and pulmonary fibroblasts [158].

Identification of A2BAR signaling as potential pathway in the pathogenesis of asthma prompted the investigation of A2BAR signaling in other chronic conditions affecting the lung, including COPD and idiopathic pulmonary fibrosis (IPF) [90,139,140,159]. The receptor is elevated in COPD patients where biopsy staining localized A2BAR expression to activated macrophages and mast cells [160,161]. Studies of IPF patients have demonstrated that expression of the receptor is increased in the remodeled airway epithelium and in the inflammatory cells [159,161]. As previously introduced, mice with partial or complete deficiency in the adenosine deaminating enzyme, ADA, experience adenosine dependant pulmonary fibrosis and have been proposed as murine models of adenosine dependant lung fibrosis [148,162]. Analysis of adenosine receptor expression revealed that ADA-deficient mice have elevated pulmonary expression of A2BAR [90,148]. Based on original findings of a protective role for A2BAR antagonists in the resolution of pulmonary inflammation and fibrotic processes [154,157,158,163], pharmacological antagonism of the A2BAR was pursued as a therapeutic strategy in ADA-deficient mice [90]. ADA-deficient mice were treated with a novel A2BAR antagonist, CVT-6883, which has high-affinity for the A2BAR and is capable of significantly inhibiting agonist induced increases in cAMP while not affecting alternative A2BAR signaling pathways [90]. Antagonist treatment resulted in decreased inflammatory infiltration into the lungs, in parallel with decreased pulmonary levels of pro-inflammatory cytokines TNF-α and IL-6 [90]. Intriguingly, a novel study with mice genetically deficient in both ADA and A2BAR was performed to support the hypothesis that A2BAR deficiency attenuates chronic pulmonary inflammation [139]. However, this study demonstrated the opposite effect, with the double knockout mice having a significantly decreased survival rate, with enhanced pulmonary inflammation and airway destruction [140]. In particular, these mice experienced excessive lung neutrophilia with loss of pulmonary barrier function [140], consistent with previous studies in a model of acute lung injury [136]. This phenotype may be due to increased levels of TNF-α in the double knockout mice [140], which have also been observed in A2BAR deficient mice following LPS exposure [69]. Mice deficient in A2BAR and ADA have enhanced airway destruction which was associated with increased damage to the alveolar airways, airway angiogenesis and collagen production [140]. The authors suggest that the enhanced lung tissue destruction may be due to the significant accumulation of pulmonary neutrophils in the double knockout mice [140]. Consistent with pharmacological studies where treatment of ADA-deficient mice with an A2BAR antagonist decreased pulmonary levels of IL-6 [90], mice lacking both ADA and A2BAR had reduced levels of IL-6 in the lung [140]. IL-6 is generally considered a pro-fibrogenic cytokine and specific regulation of pulmonary IL-6 by A2BAR may point to a critical event in the pathogenic effects of A2BAR involvement in lung fibrosis [140]. The authors explain the opposing effects observed in their pharmacological and genetic study by indicating that A2BAR signaling may be tissue protective in the acute stage of disease and progress to a pathogenic response in chronic lung inflammation [140]. However, the role for adenosine and A2BAR signaling in chronic lung disease remains controversial as mice that lack the enzyme (CD73) to generate extracellular adenosine experience enhanced pulmonary inflammation and fibrosis in a murine model of bleomycin-induced lung injury [138]. What is evident is the importance of divergent cell types in the different models, namely the alveolar macrophage in the ADA-deficient mice and the neutrophil in the A2BAR/ADA-deficient mouse indicates the potential for cell type dependent effects of A2BAR signaling. Taken together it is clear that it will be important to clarify the effect of specific targeting of the A2BAR at different time-points or on different cell types in the chronic disease process to elucidate the exact therapeutic function of A2BAR signaling.

2.7. A2BAR in intestinal inflammation

There is strong evidence to suggest that extracellular adenosine plays a protective role during intestinal inflammation [164–166]. This prompted investigation of the role for adenosine receptor signaling during intestinal inflammation. Of the four adenosine receptors, the A2BAR is specifically expressed in the gastrointestinal tract [67]. Initial studies focusing on the role of the receptor *in vitro* demonstrated that A2BAR is located on both the apical and basolateral surface of the intestinal epithelium where it can induce chloride secretion through a cAMP dependent pathway, suggesting that A2BAR could be important in the diarrheal process [67]. Cell culture studies determined that the A2BAR associates with adenylate cyclase isoform 6 in intestinal epithelial cells to mediate A2BAR-dependent increases in intracellular cAMP [167]. A recent study pointed to the physiological relevance of A2BAR expression in colonic motility [168]. Observations in mice lacking the A2BAR or mice treated with an A2BAR antagonist pointed to a phenotype similar to human constipation, with increased stool retention and delayed colonic emptying [168]. Electrophysiological studies demonstrated A2BAR agonists induce colonic relaxation by increasing levels of nitric oxide [168]. These novel *in vivo* observations indicate that specific targeting of the A2BAR could be used as a therapeutic approach for diarrhea or constipation.

A2BAR signaling has been implicated in inflammatory conditions that affect the intestine, such as Crohn's disease and ulcerative colitis that affect the small intestine and colon, respectively [54,77,169,170]. A2BAR expression is induced during intestinal ischemia reperfusion and by inflammatory mediators closely associated with inflammatory bowel diseases (2.4) [47,77]. As previously discussed, the A2BAR signaling attenuates intestinal inflammation associated with ischemia/reperfusion injury [47]. In parallel with these studies, genetic or pharmacological inhibition of A2BAR resulted in exacerbation of the acute inflammatory phase of chemically-induced colitis [54]. All parameters measured demonstrated a greater disease activity in A2BAR deficient mice, including histological damage and inflammatory cell infiltration [54].

This heightened disease phenotype was linked with a specific deficiency in IL-10 expression in mice lacking the A2BAR, indicating a regulatory role for A2BAR modulation of IL-10, a protective cytokine, in dextran sodium sulphate (DSS) induced colitis [54]. This anti-inflammatory effect of A2BAR signaling corresponds with the previously mentioned studies on intestinal ischemia–reperfusion injury [47], and studies in other models of ischemia and mucosal inflammation [50,57,136].

2.8. Existing controversies

In contrast to several studies indicating a protective role of A2BAR signaling in intestinal tissue protection and attenuation of inflammation, recent reports demonstrated a deleterious effect of A2BAR signaling in murine colitis [169,170]. The initial study depicted mice deficient in A2BAR were protected in models of colitis, linking this phenotype with a diminished release of the chemoattractant KC from colonic epithelia and therefore reduced neutrophil infiltration in A2BAR deficient mice, a common component of the pathogenic response in the models tested [170]. A second study used a novel specific A2BAR antagonist to demonstrate that blockade of A2BAR improves colitis associated disease activity [169]. A2BAR blockade was associated with attenuation of colitis-induced diarrhea and inflammatory cell infiltration [169]. Specific A2BAR signaling on the intestinal epithelium or immune cells has yet to be implicated in the protective effects seen with blockade of A2BAR [169]. Why the results from these studies differ is not yet fully understood. Potential explanations for the divergent results studies could include details in the colitis protocol, disparity in murine strains with genetic deletion of the A2BAR or differences in the ambience, including diversity in bacterial flora of the mice. Of particular interest is the use of the IL-10 knockout model in the study demonstrating protection by A2BAR blockade [169]. Previous study had linked A2BAR signaling as a protective response in murine colitis through the regulation of IL-10 expression [54]. Therefore, the lack of IL-10 in this model may explain the divergent effects observed with A2BAR blockade [54,169]. Furthermore, it seems important to point out that in a related study, A2BAR deficient mice experienced a pro-inflammatory phenotype with increased susceptibility to Salmonella infection [170]. This is in agreement with previous tissue protective effects of A2BAR signaling that have been demonstrated in many models of inflammation and ischemia [30,47,49,50,55,57,69,107,121,136]. Due to the expression of A2BAR on intestinal epithelia and immune cells, cell types which have an important interplay in inflammatory bowel diseases, it is of greatest importance to elucidate the cell specific effects of A2BAR signaling in regards to inflammatory bowel diseases.

3. Conclusion

Elucidation of a low-affinity subtype of the A2 adenosine receptors, the A2BAR, led to the intriguing possibility that there was a physiological receptor to counterbalance the high-affinity A2AAR [20]. Initial attempts to characterize the A2BAR were hampered by the lack of specific pharmacological agents to target the receptor [15,16,18,26]. The advent of more sensitive molecular techniques allowed for the detection of the A2BAR on many cell types, particularly in the vasculature, which gave initial insight into a functional role for the A2BAR. Genetic manipulation techniques have played a key role in recent elucidation of the functions of the A2BAR. Not only has this technology been used to investigate A2BAR function, but generation of an A2BAR deficient-reporter mouse has allowed for highly sensitive detection of A2BAR expression [69]. The availability of genetic models coupled with novel pharmacological tools for specific targeting of the A2BAR has significantly advanced our knowledge of the functional consequences of A2BAR signaling.

Transcriptional studies revealed that A2BAR expression is significantly upregulated in response to a variety of inflammatory mediators (2.4). Recent analysis of the A2BAR promoter revealed novel transcription factors that may modulate A2BAR expression. Analysis of these signaling pathways could be an interesting avenue of investigation that may implicate A2BAR signaling in a previously unappreciated biological role. The latest studies reveal that inflammatory mediators are

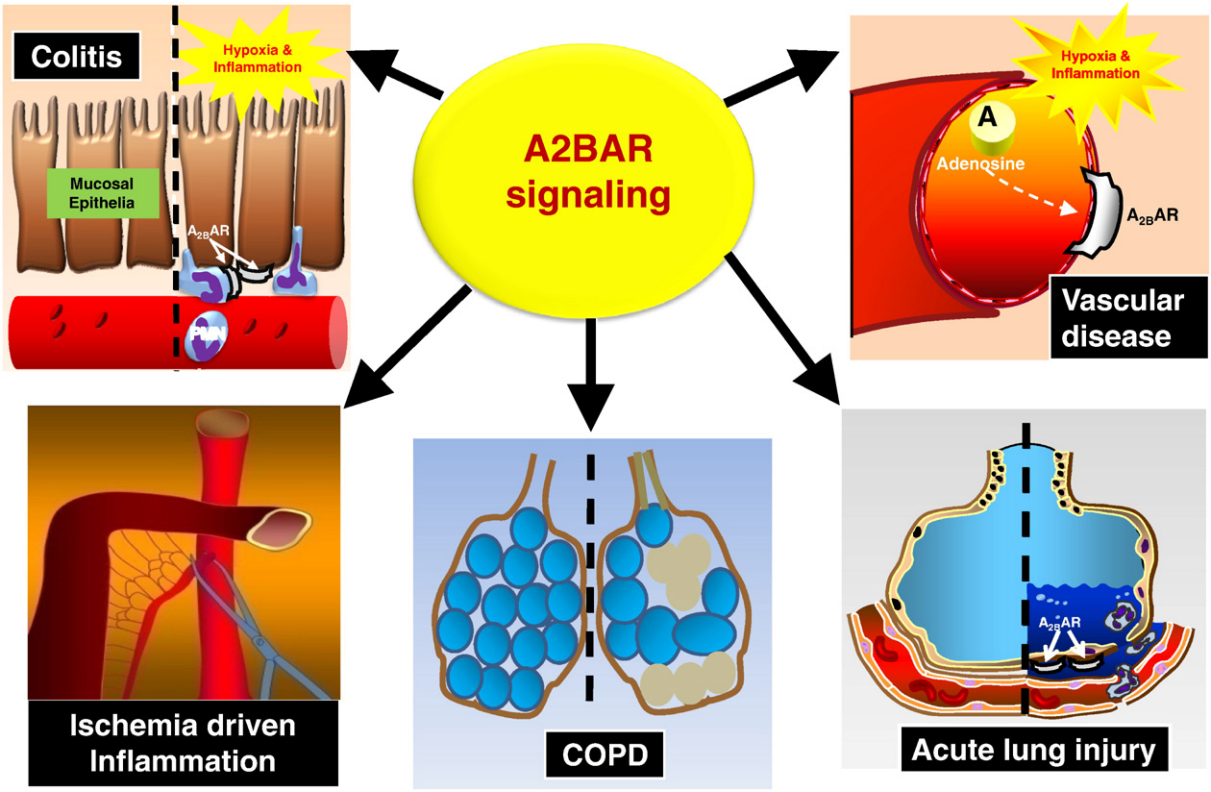

Fig. 3. A2BAR involvement in inflammatory disease.

implicated in novel regulation of the receptor by post-transcriptional mechanisms [49,80]. Further investigation of these pathways will yield novel insight into alternative methods of regulation of the A2BAR during the inflammatory disease process.

The observation that A2BAR expression is controlled by hypoxia and inflammation is significant when considering that the level of extracellular adenosine is dramatically enhanced in ischemic and inflamed tissue. This has prompted novel studies to investigate a potential role for A2BAR signaling in many models of ischemia and inflammation. The availability of mice deficient in the A2BAR and reporter mice deficient in the A2BAR has greatly facilitated these studies. Current reports demonstrate a tissue protective role for A2BAR signaling during ischemic disease or diseases characterized by acute inflammation. Expression of A2BAR on the hematopoietic cells is largely agreed to be responsible for the anti-inflammatory effect of A2BAR in these models. Development of an A2BAR specific agonist, BAY-60-6583, that has dramatically enhanced affinity for the A2BAR has provided an important pharmacological tool to elucidate the outcome of therapeutically targeting the A2BAR [30]. Application of this novel receptor agonist *in vivo* has demonstrated profound tissue protective responses with specific enhancement of A2BAR signaling in murine models of acute ischemia and inflammation. Both, genetic and pharmacological observations support the rationale for treatment with A2BAR agonists in ischemic disease. The role of A2BAR in diseases characterized by chronic inflammation, such as COPD and inflammatory bowel disease, is somewhat more controversial. Studies have demonstrated both tissue protective and deleterious effects of A2BAR signaling in models of both diseases. The involvement of A2BAR signaling on distinct cell types involved in the chronic disease process has yet to be elucidated (Fig. 3). What can be taken from these conflicting studies is that further investigation of the cell specific effect of A2BAR signaling in these models is of the utmost importance. This would allow for specific targeting of the A2BAR on the most appropriate cell types at the relevant point in the disease process where A2BAR signaling could exert its maximal therapeutic effect.

It is demonstrated by the multiple studies employing the new genetic and pharmacological tools specific for the A2BAR that the A2BAR has significantly advanced in biological importance since its humble beginnings as the low-affinity counterpart to the A2AAR. It is exciting to think what the future holds for the therapeutic potential of the A2BAR.

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References

[1] M. Kim, S.W. Chen, S.W. Park, V.D. D'Agati, J. Yang, H.T. Lee, Kidney-specific reconstitution of the A1 adenosine receptor in A1 adenosine receptor knockout mice reduces renal ischemia–reperfusion injury, Kidney Int. 75 (2009) 809–823.
[2] S.W. Park, S.W. Chen, M. Kim, K.M. Brown, V.D. D'Agati, H.T. Lee, Protection against acute kidney injury via A1 adenosine receptor-mediated Akt activation reduces liver injury after liver ischemia and reperfusion in mice, J. Pharmacol. Exp. Ther. 333 (3) (2010) 736–747.
[3] A.N. Drury, A. Szent-Gyorgyi, The physiological activity of adenine compounds with especial reference to their action upon the mammalian heart, J. Physiol. 68 (1929) 213–237.
[4] T. Eckle, M. Koeppen, H.K. Eltzschig, Role of extracellular adenosine in acute lung injury, Physiology (Bethesda) 24 (2009) 298–306.
[5] H.K. Eltzschig, Adenosine: an old drug newly discovered, Anesthesiology 111 (2009) 904–915.
[6] K. Synnestvedt, G.T. Furuta, K.M. Comerford, N. Louis, J. Karhausen, H.K. Eltzschig, K.R. Hansen, L.F. Thompson, S.P. Colgan, Ecto-5′-nucleotidase (CD73) regulation by hypoxia-inducible factor-1 mediates permeability changes in intestinal epithelia, J. Clin. Invest. 110 (2002) 993–1002.
[7] H.K. Eltzschig, J.C. Ibla, G.T. Furuta, M.O. Leonard, K.A. Jacobson, K. Enjyoji, S.C. Robson, S.P. Colgan, Coordinated adenine nucleotide phosphohydrolysis and nucleoside signaling in posthypoxic endothelium: role of ectonucleotidases and adenosine A2B receptors, J. Exp. Med. 198 (2003) 783–796.
[8] L.F. Thompson, H.K. Eltzschig, J.C. Ibla, C.J. Van De Wiele, R. Resta, J.C. Morote-Garcia, S.P. Colgan, Crucial role for ecto-5′-nucleotidase (CD73) in vascular leakage during hypoxia, J. Exp. Med. 200 (2004) 1395–1405.
[9] H.K. Eltzschig, L.F. Thompson, J. Karhausen, R.J. Cotta, J.C. Ibla, S.C. Robson, S.P. Colgan, Endogenous adenosine produced during hypoxia attenuates neutrophil accumulation: coordination by extracellular nucleotide metabolism, Blood 104 (2004) 3986–3992.
[10] L.B. Cobbin, R. Einstein, M.H. Maguire, Studies on the coronary dilator actions of some adenosine analogues, Br. J. Pharmacol. 50 (1974) 25–33.
[11] T. Degubareff, W. Sleator Jr., Effects of caffeine on mammalian atrial muscle, and its interaction with adenosine and calcium, J. Pharmacol. Exp. Ther. 148 (1965) 202–214.
[12] A. Sattin, T.W. Rall, The effect of adenosine and adenine nucleotides on the cyclic adenosine 3′, 5′-phosphate content of guinea pig cerebral cortex slices, Mol. Pharmacol. 6 (1970) 13–23.
[13] A. Burnstock, basis for distinguishing two types of purinergic receptor, in: L. Bolis, R.W. Straub (Eds.), Cell Membrane Receptors for Drugs and Hormones, Raven Press, New York, 1978, pp. 107–118.
[14] D. van Calker, M. Muller, B. Hamprecht, Adenosine regulates via two different types of receptors, the accumulation of cyclic AMP in cultured brain cells, J. Neurochem. 33 (1979) 999–1005.
[15] C. Londos, D.M. Cooper, J. Wolff, Subclasses of external adenosine receptors, Proc. Natl. Acad. Sci. U.S.A. 77 (1980) 2551–2554.
[16] J.W. Daly, P. Butts-Lamb, W. Padgett, Subclasses of adenosine receptors in the central nervous system: interaction with caffeine and related methylxanthines, Cell. Mol. Neurobiol. 3 (1983) 69–80.
[17] L. Elfman, E. Lindgren, E. Walum, B.B. Fredholm, Adenosine analogues stimulate cyclic AMP-accumulation in cultured neuroblastoma and glioma cells, Acta Pharmacol. Toxicol. (Copenh.) 55 (1984) 297–302.
[18] R.F. Bruns, G.H. Lu, T.A. Pugsley, Characterization of the A2 adenosine receptor labeled by [3H]NECA in rat striatal membranes, Mol. Pharmacol. 29 (1986) 331–346.
[19] F. Libert, M. Parmentier, A. Lefort, C. Dinsart, J. Van Sande, C. Maenhaut, M.J. Simons, J.E. Dumont, G. Vassart, Selective amplification and cloning of four new members of the G protein-coupled receptor family, Science 244 (1989) 569–572.
[20] J.H. Stehle, S.A. Rivkees, J.J. Lee, D.R. Weaver, J.D. Deeds, S.M. Reppert, Molecular cloning and expression of the cDNA for a novel A2-adenosine receptor subtype, Mol. Endocrinol. 6 (1992) 384–393.
[21] S.A. Rivkees, S.M. Reppert, RFL9 encodes an A2b-adenosine receptor, Mol. Endocrinol. 6 (1992) 1598–1604.
[22] K.D. Pierce, T.J. Furlong, L.A. Selbie, J. Shine, Molecular cloning and expression of an adenosine A2b receptor from human brain, Biochem. Biophys. Res. Commun. 187 (1992) 86–93.
[23] D.L. Marquardt, L.L. Walker, S. Heinemann, Cloning of two adenosine receptor subtypes from mouse bone marrow-derived mast cells, J. Immunol. 152 (1994) 4508–4515.
[24] M.A. Jacobson, R.G. Johnson, C.J. Luneau, C.A. Salvatore, Cloning and chromosomal localization of the human A2b adenosine receptor gene (ADORA2B) and its pseudogene, Genomics 27 (1995) 374–376.
[25] K.N. Klotz, J. Hessling, J. Hegler, C. Owman, B. Kull, B.B. Fredholm, M.J. Lohse, Comparative pharmacology of human adenosine receptor subtypes — characterization of stably transfected receptors in CHO cells, Naunyn-Schmiedebergs Arch. Pharmacol. 357 (1998) 1–9.
[26] L.E. Brackett, J.W. Daly, Functional characterization of the A2b adenosine receptor in NIH 3T3 fibroblasts, Biochem. Pharmacol. 47 (1994) 801–814.
[27] I. Feoktistov, I. Biaggioni, Pharmacological characterization of adenosine A2B receptors: studies in human mast cells co-expressing A2A and A2B adenosine receptor subtypes, Biochem. Pharmacol. 55 (1998) 627–633.
[28] I. Feoktistov, I. Biaggioni, Characterization of adenosine receptors in human erythroleukemia cells and platelets: further evidence for heterogeneity of adenosine A2 receptor subtypes, Mol. Pharmacol. 43 (1993) 909–914.
[29] G. Schulte, B.B. Fredholm, Human adenosine A(1), A(2A), A(2B), and A(3) receptors expressed in Chinese hamster ovary cells all mediate the phosphorylation of extracellular-regulated kinase 1/2, Mol. Pharmacol. 58 (2000) 477–482.
[30] T. Eckle, T. Krahn, A. Grenz, D. Kohler, M. Mittelbronn, C. Ledent, M.A. Jacobson, H. Osswald, L.F. Thompson, K. Unertl, H.K. Eltzschig, Cardioprotection by ecto-5′-nucleotidase (CD73) and A2B adenosine receptors, Circulation 115 (2007) 1581–1590.
[31] T. Eckle, L. Fullbier, M. Wehrmann, J. Khoury, M. Mittelbronn, J. Ibla, P. Rosenberger, H.K. Eltzschig, Identification of ectonucleotidases CD39 and CD73 in innate protection during acute lung injury, J. Immunol. 178 (2007) 8127–8137.
[32] M.L. Hart, M. Henn, D. Kohler, D. Kloor, M. Mittelbronn, I.C. Gorzolla, G.L. Stahl, H. K. Eltzschig, Role of extracellular nucleotide phosphohydrolysis in intestinal ischemia–reperfusion injury, FASEB J. 22 (2008) 2784–2797.
[33] M.L. Hart, C. Much, I.C. Gorzolla, J. Schittenhelm, D. Kloor, G.L. Stahl, H.K. Eltzschig, Extracellular adenosine production by ecto-5′-nucleotidase protects during murine hepatic ischemic preconditioning, Gastroenterology 135 (2008) 1739-1750 e1733.
[34] J. Reutershan, I. Vollmer, S. Stark, R. Wagner, K.C. Ngamsri, H.K. Eltzschig, Adenosine and inflammation: CD39 and CD73 are critical mediators in LPS-induced PMN trafficking into the lungs, FASEB J. 23 (2009) 473–482.
[35] H.K. Eltzschig, T. Eckle, A. Mager, N. Kuper, C. Karcher, T. Weissmuller, K. Boengler, R. Schulz, S.C. Robson, S.P. Colgan, ATP release from activated neutrophils occurs via connexin 43 and modulates adenosine-dependent endothelial cell function, Circ. Res. 99 (2006) 1100–1108.

[36] A. Grenz, H. Zhang, M. Hermes, T. Eckle, K. Klingel, D.Y. Huang, C.E. Muller, S.C. Robson, H. Osswald, H.K. Eltzschig, Contribution of E-NTPDase1 (CD39) to renal protection from ischemia–reperfusion injury, FASEB J. 21 (2007) 2863–2873.
[37] D. Kohler, T. Eckle, M. Faigle, A. Grenz, M. Mittelbronn, S. Laucher, M.L. Hart, S.C. Robson, C.E. Muller, H.K. Eltzschig, CD39/ectonucleoside triphosphate diphosphohydrolase 1 provides myocardial protection during cardiac ischemia/reperfusion injury, Circulation 116 (2007) 1784–1794.
[38] M.L. Hart, I.C. Gorzolla, J. Schittenhelm, S.C. Robson, H.K. Eltzschig, SP1-dependent induction of CD39 facilitates hepatic ischemic preconditioning, J. Immunol. 184 (7) (2010) 4017–4024.
[39] H.K. Eltzschig, P. Abdulla, E. Hoffman, K.E. Hamilton, D. Daniels, C. Schonfeld, M. Loffler, G. Reyes, M. Duszenko, J. Karhausen, A. Robinson, K.A. Westerman, I.R. Coe, S.P. Colgan, HIF-1-dependent repression of equilibrative nucleoside transporter (ENT) in hypoxia, J. Exp. Med. 202 (2005) 1493–1505.
[40] M. Loffler, J.C. Morote-Garcia, S.A. Eltzschig, I.R. Coe, H.K. Eltzschig, Physiological roles of vascular nucleoside transporters, Arterioscler. Thromb. Vasc. Biol. 27 (2007) 1004–1013.
[41] J.C. Morote-Garcia, P. Rosenberger, J. Kuhlicke, H.K. Eltzschig, HIF-1-dependent repression of adenosine kinase attenuates hypoxia-induced vascular leak, Blood 111 (2008) 5571–5580.
[42] J.C. Morote-Garcia, P. Rosenberger, N.M. Nivillac, I.R. Coe, H.K. Eltzschig, Hypoxia-inducible factor-dependent repression of equilibrative nucleoside transporter 2 attenuates mucosal inflammation during intestinal hypoxia, Gastroenterology 136 (2009) 607–618.
[43] G. Hasko, B. Csoka, Z.H. Nemeth, E.S. Vizi, P. Pacher, A(2B) adenosine receptors in immunity and inflammation, Trends Immunol. 30 (6) (2009) 263–270.
[44] G. Hasko, J. Linden, B. Cronstein, P. Pacher, Adenosine receptors: therapeutic aspects for inflammatory and immune diseases, Nat. Rev. Drug Discov. 7 (2008) 759–770.
[45] H.K. Eltzschig, D. Kohler, T. Eckle, T. Kong, S.C. Robson, S.P. Colgan, Central role of Sp1-regulated CD39 in hypoxia/ischemia protection, Blood 113 (2009) 224–232.
[46] M.L. Hart, I.C. Gorzolla, J. Schittenhelm, S.C. Robson, H.K. Eltzschig, SP1-dependent induction of CD39 facilitates hepatic ischemic preconditioning, J. Immunol. 184 (2010) 4017–4024.
[47] M.L. Hart, B. Jacobi, J. Schittenhelm, M. Henn, H.K. Eltzschig, Cutting edge: A2B adenosine receptor signaling provides potent protection during intestinal ischemia/reperfusion injury, J. Immunol. 182 (2009) 3965–3968.
[48] T. Kong, K.A. Westerman, M. Faigle, H.K. Eltzschig, S.P. Colgan, HIF-dependent induction of adenosine A2B receptor in hypoxia, FASEB J. 20 (2006) 2242–2250.
[49] U. Schingnitz, K. Hartman, C.F. MacManus, T. Eckle, S. Zug, S.P. Colgan, H.K. Eltzschig, Signaling through the A2B adenosine receptor dampens endotoxin-induced acute lung injury, J. Immunol. 184 (9) (2010) 5271–5279.
[50] T. Eckle, M. Faigle, A. Grenz, S. Laucher, L.F. Thompson, H.K. Eltzschig, A2B adenosine receptor dampens hypoxia-induced vascular leak, Blood 111 (2008) 2024–2035.
[51] A. Grenz, H. Osswald, T. Eckle, D. Yang, H. Zhang, Z.V. Tran, K. Klingel, K. Ravid, H.K. Eltzschig, The reno-vascular A2B adenosine receptor protects the kidney from ischemia, PLoS Med. 5 (2008) e137.
[52] T. Eckle, D. Kohler, R. Lehmann, K.C. El Kasmi, H.K. Eltzschig, Hypoxia-inducible factor-1 is central to cardioprotection: a new paradigm for ischemic preconditioning, Circulation 118 (2008) 166–175.
[53] T. Eckle, A. Grenz, S. Laucher, H.K. Eltzschig, A2B adenosine receptor signaling attenuates acute lung injury by enhancing alveolar fluid clearance in mice, J. Clin. Invest. 118 (2008) 3301–3315.
[54] J.S. Frick, C.F. MacManus, M. Scully, L.E. Glover, H.K. Eltzschig, S.P. Colgan, Contribution of adenosine A2B receptors to inflammatory parameters of experimental colitis, J. Immunol. 182 (2009) 4957–4964.
[55] T. Eckle, D. Kohler, R. Lehmann, K. El Kasmi, H.K. Eltzschig, Hypoxia-inducible factor-1 is central to cardioprotection: a new paradigm for ischemic preconditioning, Circulation 118 (2008) 166–175.
[56] H.K. Eltzschig, J. Rivera-Nieves, S.P. Colgan, Targeting the A2B adenosine receptor during gastrointestinal ischemia and inflammation, Expert Opin. Ther. Targets 13 (2009) 1267–1277.
[57] P. Rosenberger, J.M. Schwab, V. Mirakaj, E. Masekowsky, A. Mager, J.C. Morote-Garcia, K. Unertl, H.K. Eltzschig, Hypoxia-inducible factor-dependent induction of netrin-1 dampens inflammation caused by hypoxia, Nat. Immunol. 10 (2009) 195–202.
[58] I. Feoktistov, I. Biaggioni, Adenosine A2b receptors evoke interleukin-8 secretion in human mast cells. An enprofylline-sensitive mechanism with implications for asthma, J. Clin. Invest. 96 (1995) 1979–1986.
[59] J. Xaus, M. Mirabet, J. Lloberas, C. Soler, C. Lluis, R. Franco, A. Celada, IFN-gamma up-regulates the A2B adenosine receptor expression in macrophages: a mechanism of macrophage deactivation, J. Immunol. 162 (1999) 3607–3614.
[60] M. Mirabet, C. Herrera, O.J. Cordero, J. Mallol, C. Lluis, R. Franco, Expression of A2B adenosine receptors in human lymphocytes: their role in T cell activation, J. Cell Sci. 112 (Pt 4) (1999) 491–502.
[61] Y. Zhang, J. Raud, P. Hedqvist, B.B. Fredholm, Propentofylline inhibits polymorphonuclear leukocyte recruitment in vivo by a mechanism involving adenosine A2A receptors, Eur. J. Pharmacol. 313 (1996) 237–242.
[62] S.V. Novitskiy, S. Ryzhov, R. Zaynagetdinov, A.E. Goldstein, Y. Huang, O.Y. Tikhomirov, M.R. Blackburn, I. Biaggioni, D.P. Carbone, I. Feoktistov, M.M. Dikov, Adenosine receptors in regulation of dendritic cell differentiation and function, Blood 112 (2008) 1822–1831.
[63] A. Ben Addi, A. Lefort, X. Hua, F. Libert, D. Communi, C. Ledent, P. Macours, S.L. Tilley, J.M. Boeynaems, B. Robaye, Modulation of murine dendritic cell function by adenine nucleotides and adenosine: involvement of the A(2B) receptor, Eur. J. Immunol. 38 (2008) 1610–1620.
[64] I. Feoktistov, A.E. Goldstein, S. Ryzhov, D. Zeng, L. Belardinelli, T. Voyno-Yasenetskaya, I. Biaggioni, Differential expression of adenosine receptors in human endothelial cells: role of A2B receptors in angiogenic factor regulation, Circ. Res. 90 (2002) 531–538.
[65] B.T. Liang, B. Haltiwanger, Adenosine A2a and A2b receptors in cultured fetal chick heart cells. High- and low-affinity coupling to stimulation of myocyte contractility and cAMP accumulation, Circ Res 76 (1995) 242–251.
[66] C.Y. Gregory, T.A. Abrams, M.O. Hall, Stimulation of A2 adenosine receptors inhibits the ingestion of photoreceptor outer segments by retinal pigment epithelium, Invest. Ophthalmol. Vis. Sci. 35 (1994) 819–825.
[67] G.R. Strohmeier, S.M. Reppert, W.I. Lencer, J.L. Madara, The A2b adenosine receptor mediates cAMP responses to adenosine receptor agonists in human intestinal epithelia, J. Biol. Chem. 270 (1995) 2387–2394.
[68] R.E. Cagnina, S.I. Ramos, M.A. Marshall, G. Wang, C.R. Frazier, J. Linden, Adenosine A2B receptors are highly expressed on murine type II alveolar epithelial cells, Am. J. Physiol. Lung Cell. Mol. Physiol. 297 (2009) L467–L474.
[69] D. Yang, Y. Zhang, H.G. Nguyen, M. Koupenova, A.K. Chauhan, M. Makitalo, M.R. Jones, C. St Hilaire, D.C. Seldin, P. Toselli, E. Lamperti, B.M. Schreiber, H. Gavras, D.D. Wagner, K. Ravid, The A2B adenosine receptor protects against inflammation and excessive vascular adhesion, J. Clin. Invest. 116 (2006) 1913–1923.
[70] G.L. Semenza, Life with oxygen, Science 318 (2007) 62–64.
[71] C. St Hilaire, S.H. Carroll, H. Chen, K. Ravid, Mechanisms of induction of adenosine receptor genes and its functional significance, J. Cell. Physiol. 218 (2009) 35–44.
[72] H. Akazawa, I. Komuro, Cardiac transcription factor Csx/Nkx2-5: its role in cardiac development and diseases, Pharmacol. Ther. 107 (2005) 252–268.
[73] H. Toko, W. Zhu, E. Takimoto, I. Shiojima, Y. Hiroi, Y. Zou, T. Oka, H. Akazawa, M. Mizukami, M. Sakamoto, F. Terasaki, Y. Kitaura, H. Takano, T. Nagai, R. Nagai, I. Komuro, Csx/Nkx2-5 is required for homeostasis and survival of cardiac myocytes in the adult heart, J. Biol. Chem. 277 (2002) 24735–24743.
[74] A. Kuno, S.D. Critz, L. Cui, V. Solodushko, X.M. Yang, T. Krahn, B. Albrecht, S. Philipp, M.V. Cohen, J.M. Downey, Protein kinase C protects preconditioned rabbit hearts by increasing sensitivity of adenosine A2b-dependent signaling during early reperfusion, J. Mol. Cell. Cardiol. 43 (2007) 262–271.
[75] Z.H. Nemeth, S.J. Leibovich, E.A. Deitch, E.S. Vizi, C. Szabo, G. Hasko, cDNA microarray analysis reveals a nuclear factor-kappaB-independent regulation of macrophage function by adenosine, J. Pharmacol. Exp. Ther. 306 (2003) 1042–1049.
[76] C. St Hilaire, M. Koupenova, S.H. Carroll, B.D. Smith, K. Ravid, TNF-alpha upregulates the A2B adenosine receptor gene: the role of NAD(P)H oxidase 4, Biochem. Biophys. Res. Commun. 375 (2008) 292–296.
[77] V. Kolachala, V. Asamoah, L. Wang, T.S. Obertone, T.R. Ziegler, D. Merlin, S.V. Sitaraman, TNF-alpha upregulates adenosine 2b (A2b) receptor expression and signaling in intestinal epithelial cells: a basis for A2bR overexpression in colitis, Cell. Mol. Life Sci. 62 (2005) 2647–2657.
[78] D.K. Nguyen, M.C. Montesinos, A.J. Williams, M. Kelly, B.N. Cronstein, Th1 cytokines regulate adenosine receptors and their downstream signaling elements in human microvascular endothelial cells, J. Immunol. 171 (2003) 3991–3998.
[79] V. Kolachala, V. Asamoah, L. Wang, S. Srinivasan, D. Merlin, S.V. Sitaraman, Interferon-gamma down-regulates adenosine 2b receptor-mediated signaling and short circuit current in the intestinal epithelia by inhibiting the expression of adenylate cyclase, J. Biol. Chem. 280 (2005) 4048–4057.
[80] V.L. Kolachala, L. Wang, T.S. Obertone, M. Prasad, Y. Yan, G. Dalmasso, A. Gewirtz, D. Merlin, S.V. Sitaraman, Adenosine 2B receptor expression is post-transcriptionally regulated by microRNA, J. Biol. Chem. (2010). [Electronic publication ahead of print].
[81] L. Wang, V. Kolachala, B. Walia, S. Balasubramanian, R.A. Hall, D. Merlin, S.V. Sitaraman, Agonist-induced polarized trafficking and surface expression of the adenosine 2b receptor in intestinal epithelial cells: role of SNARE proteins, Am. J. Physiol. Gastrointest. Liver Physiol. 287 (2004) G1100–G1107.
[82] S.V. Sitaraman, L. Wang, M. Wong, M. Bruewer, M. Hobert, C.H. Yun, D. Merlin, J.L. Madara, The adenosine 2b receptor is recruited to the plasma membrane and associates with E3KARP and Ezrin upon agonist stimulation, J. Biol. Chem. 277 (2002) 33188–33195.
[83] B.B. Fredholm, E. Irenius, B. Kull, G. Schulte, Comparison of the potency of adenosine as an agonist at human adenosine receptors expressed in Chinese hamster ovary cells, Biochem. Pharmacol. 61 (2001) 443–448.
[84] A.A. Ivanov, I.I. Baskin, V.A. Palyulin, L. Piccagli, P.G. Baraldi, N.S. Zefirov, Molecular modeling and molecular dynamics simulation of the human A2B adenosine receptor. The study of the possible binding modes of the A2B receptor antagonists, J. Med. Chem. 48 (2005) 6813–6820.
[85] A.A. Ivanov, I.I. Baskin, V.A. Palyulin, N.S. Zefirov, Molecular modeling the human A1 adenosine receptor and study of the mechanisms of its selective ligand binding, Dokl. Biochem. Biophys. 386 (2002) 271–274.
[86] F.F. Sherbiny, A.C. Schiedel, A. Maass, C.E. Muller, Homology modelling of the human adenosine A2B receptor based on X-ray structures of bovine rhodopsin, the beta2-adrenergic receptor and the human adenosine A2A receptor, J. Comput. Aided Mol. Des. 23 (2009) 807–828.
[87] K. Palczewski, T. Kumasaka, T. Hori, C.A. Behnke, H. Motoshima, B.A. Fox, I. Le Trong, D.C. Teller, T. Okada, R.E. Stenkamp, M. Yamamoto, M. Miyano, Crystal structure of rhodopsin: a G protein-coupled receptor, Science 289 (2000) 739–745.
[88] M.W. Beukers, H. den Dulk, E.W. van Tilburg, J. Brouwer, A.P. Ijzerman, Why are A (2B) receptors low-affinity adenosine receptors? Mutation of Asn273 to Tyr increases affinity of human A(2B) receptor for 2-(1-Hexynyl)adenosine, Mol. Pharmacol. 58 (2000) 1349–1356.

[89] M.W. Beukers, J. van Oppenraaij, P.P. van der Hoorn, C.C. Blad, H. den Dulk, J. Brouwer, I.J. AP, Random mutagenesis of the human adenosine A2B receptor followed by growth selection in yeast. Identification of constitutively active and gain of function mutations, Mol. Pharmacol. 65 (2004) 702–710.
[90] C.X. Sun, H. Zhong, A. Mohsenin, E. Morschl, J.L. Chunn, J.G. Molina, L. Belardinelli, D. Zeng, M.R. Blackburn, Role of A2B adenosine receptor signaling in adenosine-dependent pulmonary inflammation and injury, J. Clin. Invest. 116 (2006) 2173–2182.
[91] I. Feoktistov, I. Biaggioni, Adenosine A2B receptors, Pharmacol. Rev. 49 (1997) 381–402.
[92] I. Feoktistov, J.J. Murray, I. Biaggioni, Positive modulation of intracellular Ca2+ levels by adenosine A2b receptors, prostacyclin, and prostaglandin E1 via a cholera toxin-sensitive mechanism in human erythroleukemia cells, Mol. Pharmacol. 45 (1994) 1160–1167.
[93] J. Linden, T. Thai, H. Figler, X. Jin, A.S. Robeva, Characterization of human A(2B) adenosine receptors: radioligand binding, western blotting, and coupling to G(q) in human embryonic kidney 293 cells and HMC-1 mast cells, Mol. Pharmacol. 56 (1999) 705–713.
[94] J. Linden, J.A. Auchampach, X. Jin, R.A. Figler, The structure and function of A1 and A2B adenosine receptors, Life Sci. 62 (1998) 1519–1524.
[95] I. Feoktistov, A.E. Goldstein, I. Biaggioni, Role of p38 mitogen-activated protein kinase and extracellular signal-regulated protein kinase kinase in adenosine A2B receptor-mediated interleukin-8 production in human mast cells, Mol. Pharmacol. 55 (1999) 726–734.
[96] Z. Gao, T. Chen, M.J. Weber, J. Linden, A2B adenosine and P2Y2 receptors stimulate mitogen-activated protein kinase in human embryonic kidney-293 cells. Cross-talk between cyclic AMP and protein kinase c pathways, J. Biol. Chem. 274 (1999) 5972–5980.
[97] G. Schulte, B.B. Fredholm, The G(s)-coupled adenosine A(2B) receptor recruits divergent pathways to regulate ERK1/2 and p38, Exp. Cell Res. 290 (2003) 168–176.
[98] S. Latini, F. Bordoni, F. Pedata, R. Corradetti, Extracellular adenosine concentrations during in vitro ischaemia in rat hippocampal slices, Br. J. Pharmacol. 127 (1999) 729–739.
[99] K.A. Rudolphi, P. Schubert, F.E. Parkinson, B.B. Fredholm, Adenosine and brain ischemia, Cerebrovasc. Brain Metab. Rev. 4 (1992) 346–369.
[100] J.L. Balwierczak, R. Sharif, C.M. Krulan, F.P. Field, G.B. Weiss, M.J. Miller, Comparative effects of a selective adenosine A2 receptor agonist, CGS 21680, and nitroprusside in vascular smooth muscle, Eur. J. Pharmacol. 196 (1991) 117–123.
[101] J. Haynes Jr., B. Obiako, W.J. Thompson, J. Downey, Adenosine-induced vasodilation: receptor characterization in pulmonary circulation, Am. J. Physiol. 268 (1995) H1862–H1868.
[102] N. Levens, M. Beil, R. Schulz, Intrarenal actions of the new adenosine agonist CGS 21680A, selective for the A2 receptor, J. Pharmacol. Exp. Ther. 257 (1991) 1013–1019.
[103] P.L. Martin, Relative agonist potencies of C2-substituted analogues of adenosine: evidence for adenosine A2B receptors in the guinea pig aorta, Eur. J. Pharmacol. 216 (1992) 235–242.
[104] R.K. Dubey, D.G. Gillespie, K. Osaka, F. Suzuki, E.K. Jackson, Adenosine inhibits growth of rat aortic smooth muscle cells. Possible role of A2b receptor, Hypertension 27 (1996) 786–793.
[105] R.K. Dubey, D.G. Gillespie, H. Shue, E.K. Jackson, A(2B) receptors mediate antimitogenesis in vascular smooth muscle cells, Hypertension 35 (2000) 267–272.
[106] M.B. Grant, R.W. Tarnuzzer, S. Caballero, M.J. Ozeck, M.I. Davis, P.E. Spoerri, I. Feoktistov, I. Biaggioni, J.C. Shryock, L. Belardinelli, Adenosine receptor activation induces vascular endothelial growth factor in human retinal endothelial cells, Circ. Res. 85 (1999) 699–706.
[107] D. Yang, M. Koupenova, D.J. McCrann, K.J. Kopeikina, H.M. Kagan, B.M. Schreiber, K. Ravid, The A2b adenosine receptor protects against vascular injury, Proc. Natl. Acad. Sc.i U.S.A. 105 (2008) 792–796.
[108] D. Yang, H. Chen, M. Koupenova-Zamor, S.H. Carroll, A. Eliades, J.E. Freedman, P. Toselli, K. Ravid, A new role for the A2b adenosine receptor in regulating platelet function, J. Thromb. Haemost. 8 (4) (2010) 817–827.
[109] J.A. Cooper, S.J. Hill, S.P. Alexander, P.C. Rubin, E.H. Horn, Adenosine receptor-induced cyclic AMP generation and inhibition of 5-hydroxytryptamine release in human platelets, Br. J. Clin. Pharmacol. 40 (1995) 43–50.
[110] K. Varani, F. Portaluppi, S. Merighi, E. Ongini, L. Belardinelli, P.A. Borea, Caffeine alters A2A adenosine receptors and their function in human platelets, Circulation 99 (1999) 2499–2502.
[111] M.D. Linden, M.R. Barnard, A.L. Frelinger, A.D. Michelson, K. Przyklenk, Effect of adenosine A2 receptor stimulation on platelet activation-aggregation: differences between canine and human models, Thromb. Res. 121 (2008) 689–698.
[112] S. Amisten, O.O. Braun, A. Bengtsson, D. Erlinge, Gene expression profiling for the identification of G-protein coupled receptors in human platelets, Thromb. Res. 122 (2008) 47–57.
[113] H.K. Eltzschig, C.F. Macmanus, S.P. Colgan, Neutrophils as sources of extracellular nucleotides: functional consequences at the vascular interface, Trends Cardiovasc. Med. 18 (2008) 103–107.
[114] M. Katori, R.M. Berne, Release of adenosine from anoxic hearts. Relationship to coronary flow, Circ. Res. 19 (1966) 420–425.
[115] R.M. Mentzer Jr., R. Rubio, R.M. Berne, Release of adenosine by hypoxic canine lung tissue and its possible role in pulmonary circulation, Am. J. Physiol. 229 (1975) 1625–1631.
[116] J. Schrader, F.J. Haddy, E. Gerlach, Release of adenosine, inosine and hypoxanthine from the isolated guinea pig heart during hypoxia, flow-autoregulation and reactive hyperemia, Pflugers Arch. 369 (1977) 1–6.
[117] M. Thiel, A. Chouker, A. Ohta, E. Jackson, C. Caldwell, P. Smith, D. Lukashev, I. Bittmann, M.V. Sitkovsky, Oxygenation inhibits the physiological tissue-protecting mechanism and thereby exacerbates acute inflammatory lung injury, PLoS Biol. 3 (2005) e174.
[118] M. Sitkovsky, D. Lukashev, Regulation of immune cells by local-tissue oxygen tension: HIF1 alpha and adenosine receptors, Nat. Rev. Immunol. 5 (2005) 712–721.
[119] M.V. Sitkovsky, D. Lukashev, S. Apasov, H. Kojima, M. Koshiba, C. Caldwell, A. Ohta, M. Thiel, Physiological control of immune response and inflammatory tissue damage by hypoxia-inducible factors and adenosine A2A receptors, Annu. Rev. Immunol. 22 (2004) 657–682.
[120] C.E. Murry, R.B. Jennings, K.A. Reimer, Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium, Circulation 74 (1986) 1124–1136.
[121] A. Grenz, H. Osswald, T. Eckle, D. Yang, H. Zhang, Z.V. Tran, K. Klingel, K. Ravid, H.K. Eltzschig, The reno-vascular A2B adenosine receptor protects the kidney from ischemia, PLoS Med. 5 (2008) e137.
[122] J. Khoury, J.C. Ibla, A.S. Neish, S.P. Colgan, Antiinflammatory adaptation to hypoxia through adenosine-mediated cullin-1 deneddylation, J. Clin. Invest. 117 (2007) 703–711.
[123] V. Corset, K.T. Nguyen-Ba-Charvet, C. Forcet, E. Moyse, A. Chedotal, P. Mehlen, Netrin-1-mediated axon outgrowth and cAMP production requires interaction with adenosine A2b receptor, Nature 407 (2000) 747–750.
[124] R.W. Schrier, W. Wang, Acute renal failure and sepsis, N. Engl. J. Med. 351 (2004) 159–169.
[125] J.G. Abuelo, Normotensive ischemic acute renal failure, N. Engl. J. Med. 357 (2007) 797–805.
[126] G.M. Chertow, E. Burdick, M. Honour, J.V. Bonventre, D.W. Bates, Acute kidney injury, mortality, length of stay, and costs in hospitalized patients, J. Am. Soc. Nephrol. 16 (2005) 3365–3370.
[127] T. Bove, G. Landoni, M.G. Calabro, G. Aletti, G. Marino, E. Cerchierini, G. Crescenzi, A. Zangrillo, Renoprotective action of fenoldopam in high-risk patients undergoing cardiac surgery: a prospective, double-blind, randomized clinical trial, Circulation 111 (2005) 3230–3235.
[128] A. Grenz, T. Eckle, H. Zhang, D.Y. Huang, M. Wehrmann, C. Kohle, K. Unertl, H. Osswald, H.K. Eltzschig, Use of a hanging-weight system for isolated renal artery occlusion during ischemic preconditioning in mice, Am. J. Physiol. Ren. Physiol. 292 (2007) F475–F485.
[129] A. Grenz, H. Zhang, T. Eckle, M. Mittelbronn, M. Wehrmann, C. Kohle, D. Kloor, L.F. Thompson, H. Osswald, H.K. Eltzschig, Protective role of ecto-5′-nucleotidase (CD73) in renal ischemia, J. Am. Soc. Nephrol. 18 (2007) 833–845.
[130] H.T. Lee, G. Gallos, S.H. Nasr, C.W. Emala, A1 adenosine receptor activation inhibits inflammation, necrosis, and apoptosis after renal ischemia–reperfusion injury in mice, J. Am. Soc. Nephrol. 15 (2004) 102–111.
[131] Y.J. Day, L. Huang, M.J. McDuffie, D.L. Rosin, H. Ye, J.F. Chen, M.A. Schwarzschild, J.S. Fink, J. Linden, M.D. Okusa, Renal protection from ischemia mediated by A2A adenosine receptors on bone marrow-derived cells, J. Clin. Invest. 112 (2003) 883–891.
[132] Y.J. Day, L. Huang, H. Ye, L. Li, J. Linden, M.D. Okusa, Renal ischemia–reperfusion injury and adenosine 2A receptor-mediated tissue protection: the role of CD4+ T cells and IFN-gamma, J. Immunol. 176 (2006) 3108–3114.
[133] L.B. Ware, M.A. Matthay, The acute respiratory distress syndrome, N. Engl. J. Med. 342 (2000) 1334–1349.
[134] T.R. Martin, Neutrophils and lung injury: getting it right, J. Clin. Invest. 110 (2002) 1603–1605.
[135] J. Henes, M.A. Schmit, J.C. Morote-Garcia, V. Mirakaj, D. Kohler, L. Glover, T. Eldh, U. Walter, J. Karhausen, S.P. Colgan, P. Rosenberger, Inflammation-associated repression of vasodilator-stimulated phosphoprotein (VASP) reduces alveolar-capillary barrier function during acute lung injury, FASEB J. 23 (2009) 4244–4255.
[136] T. Eckle, A. Grenz, S. Laucher, H.K. Eltzschig, A2B adenosine receptor signaling attenuates acute lung injury by enhancing alveolar fluid clearance in mice, J. Clin. Invest. 118 (2008) 3301–3315.
[137] D.J. Schneider, J.C. Lindsay, Y. Zhou, J.G. Molina, M.R. Blackburn, Adenosine and osteopontin contribute to the development of chronic obstructive pulmonary disease, FASEB J. 24 (2010) 70–80.
[138] J.B. Volmer, L.F. Thompson, M.R. Blackburn, Ecto-5′-nucleotidase (CD73)-mediated adenosine production is tissue protective in a model of bleomycin-induced lung injury, J. Immunol. 176 (2006) 4449–4458.
[139] R. Zaynagetdinov, S. Ryzhov, A.E. Goldstein, H. Yin, S.V. Novitskiy, K. Goleniewska, V.V. Polosukhin, D.C. Newcomb, D. Mitchell, E. Morschl, Y. Zhou, M.R. Blackburn, R.S. Peebles Jr, I. Biaggioni, I. Feoktistov, Attenuation of chronic pulmonary inflammation in A2B adenosine receptor knockout mice, Am. J. Respir. Cell Mol. Biol. 42 (5) (2010) 564–571.
[140] Y. Zhou, A. Mohsenin, E. Morschl, H.W. Young, J.G. Molina, W. Ma, C.X. Sun, H. Martinez-Valdez, M.R. Blackburn, Enhanced airway inflammation and remodeling in adenosine deaminase-deficient mice lacking the A2B adenosine receptor, J. Immunol. 182 (2009) 8037–8046.
[141] E. Huszar, G. Vass, E. Vizi, Z. Csoma, E. Barat, G. Molnar Vilagos, I. Herjavecz, I. Horvath, Adenosine in exhaled breath condensate in healthy volunteers and in patients with asthma, Eur. Respir. J. 20 (2002) 1393–1398.
[142] A.G. Driver, C.A. Kukoly, S. Ali, S.J. Mustafa, Adenosine in bronchoalveolar lavage fluid in asthma, Am. Rev. Respir. Dis. 148 (1993) 91–97.
[143] M.J. Cushley, A.E. Tattersfield, S.T. Holgate, Inhaled adenosine and guanosine on airway resistance in normal and asthmatic subjects, Br. J. Clin. Pharmacol. 15 (1983) 161–165.

[144] R.M. Senior, S.D. Shapiro, Chronic obstructive pulmonary disease: epidemiology, pathophysiology, and pathogenesis, Fishman's Pulmonary Diseases and Disorders, McGraw-Hill, New York, 1998 3.
[145] M.R. Blackburn, J.B. Volmer, J.L. Thrasher, H. Zhong, J.R. Crosby, J.J. Lee, R.E. Kellems, Metabolic consequences of adenosine deaminase deficiency in mice are associated with defects in alveogenesis, pulmonary inflammation, and airway obstruction, J. Exp. Med. 192 (2000) 159–170.
[146] H. Zhong, J.L. Chunn, J.B. Volmer, J.R. Fozard, M.R. Blackburn, Adenosine-mediated mast cell degranulation in adenosine deaminase-deficient mice, J. Pharmacol. Exp. Ther. 298 (2001) 433–440.
[147] J.L. Chunn, H.W. Young, S.K. Banerjee, G.N. Colasurdo, M.R. Blackburn, Adenosine-dependent airway inflammation and hyperresponsiveness in partially adenosine deaminase-deficient mice, J. Immunol. 167 (2001) 4676–4685.
[148] J.L. Chunn, A. Mohsenin, H.W. Young, C.G. Lee, J.A. Elias, R.E. Kellems, M.R. Blackburn, Partially adenosine deaminase-deficient mice develop pulmonary fibrosis in association with adenosine elevations, Am. J. Physiol. Lung Cell. Mol. Physiol. 290 (2006) L579–L587.
[149] M.R. Blackburn, M. Aldrich, J.B. Volmer, W. Chen, H. Zhong, S. Kelly, M.S. Hershfield, S.K. Datta, R.E. Kellems, The use of enzyme therapy to regulate the metabolic and phenotypic consequences of adenosine deaminase deficiency in mice. Differential impact on pulmonary and immunologic abnormalities, J .Biol. Chem. 275 (2000) 32114–32121.
[150] N. Crimi, F. Palermo, R. Oliveri, C. Maccarrone, B. Palermo, C. Vancheri, R. Polosa, A. Mistretta, Enhancing effect of dipyridamole inhalation on adenosine-induced bronchospasm in asthmatic patients, Allergy 43 (1988) 179–183.
[151] J.S. Mann, S.T. Holgate, Specific antagonism of adenosine-induced bronchoconstriction in asthma by oral theophylline, Br. J. Clin. Pharmacol. 19 (1985) 685–692.
[152] H. Clarke, M.J. Cushley, C.G. Persson, S.T. Holgate, The protective effects of intravenous theophylline and enprofylline against histamine- and adenosine 5′-monophosphate-provoked bronchoconstriction: implications for the mechanisms of action of xanthine derivatives in asthma, Pulm. Pharmacol. 2 (1989) 147–154.
[153] P. Bradding, A.F. Walls, S.T. Holgate, The role of the mast cell in the pathophysiology of asthma, J. Allergy Clin. Immunol. 117 (2006) 1277–1284.
[154] S. Ryzhov, A.E. Goldstein, I. Biaggioni, I. Feoktistov, Cross-talk between G(s)- and G(q)-coupled pathways in regulation of interleukin-4 by A(2B) adenosine receptors in human mast cells, Mol. Pharmacol. 70 (2006) 727–735.
[155] H. Zhong, Y. Wu, L. Belardinelli, D. Zeng, A2B adenosine receptors induce IL-19 from bronchial epithelial cells, resulting in TNF-alpha increase, Am. J. Respir. Cell Mol. Biol. 35 (2006) 587–592.
[156] H. Zhong, L. Belardinelli, T. Maa, I. Feoktistov, I. Biaggioni, D. Zeng, A(2B) adenosine receptors increase cytokine release by bronchial smooth muscle cells, Am. J. Respir. Cell Mol. Biol. 30 (2004) 118–125.
[157] S. Ryzhov, R. Zaynagetdinov, A.E. Goldstein, S.V. Novitskiy, M.R. Blackburn, I. Biaggioni, I. Feoktistov, Effect of A2B adenosine receptor gene ablation on adenosine-dependent regulation of proinflammatory cytokines, J. Pharmacol. Exp. Ther. 324 (2008) 694–700.
[158] H. Zhong, L. Belardinelli, T. Maa, D. Zeng, Synergy between A2B adenosine receptors and hypoxia in activating human lung fibroblasts, Am. J. Respir. Cell Mol. Biol. 32 (2005) 2–8.
[159] Y. Zhou, J.N. Murthy, D. Zeng, L. Belardinelli, M.R. Blackburn, Alterations in adenosine metabolism and signaling in patients with chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis, PLoS ONE 5 (2010) e9224.
[160] K. Varani, G. Caramori, F. Vincenzi, I. Adcock, P. Casolari, E. Leung, S. Maclennan, S. Gessi, S. Morello, P.J. Barnes, K. Ito, K.F. Chung, G. Cavallesco, G. Azzena, A. Papi, P.A. Borea, Alteration of adenosine receptors in patients with chronic obstructive pulmonary disease, Am. J. Respir. Crit. Care Med. 173 (2006) 398–406.
[161] M. Selman, G. Carrillo, A. Estrada, M. Mejia, C. Becerril, J. Cisneros, M. Gaxiola, R. Perez-Padilla, C. Navarro, T. Richards, J. Dauber, T.E. King Jr., A. Pardo, N. Kaminski, Accelerated variant of idiopathic pulmonary fibrosis: clinical behavior and gene expression pattern, PLoS ONE 2 (2007) e482.
[162] J.L. Chunn, J.G. Molina, T. Mi, Y. Xia, R.E. Kellems, M.R. Blackburn, Adenosine-dependent pulmonary fibrosis in adenosine deaminase-deficient mice, J. Immunol. 175 (2005) 1937–1946.
[163] S. Ryzhov, A.E. Goldstein, A. Matafonov, D. Zeng, I. Biaggioni, I. Feoktistov, Adenosine-activated mast cells induce IgE synthesis by B lymphocytes: an A2B-mediated process involving Th2 cytokines IL-4 and IL-13 with implications for asthma, J. Immunol. 172 (2004) 7726–7733.
[164] N.A. Louis, A.M. Robinson, C.F. MacManus, J. Karhausen, M. Scully, S.P. Colgan, Control of IFN-alphaA by CD73: implications for mucosal inflammation, J. Immunol. 180 (2008) 4246–4255.
[165] D.J. Friedman, B.M. Kunzli, A.R. YI, J. Sevigny, P.O. Berberat, K. Enjyoji, E. Csizmadia, H. Friess, S.C. Robson, From the cover: CD39 deletion exacerbates experimental murine colitis and human polymorphisms increase susceptibility to inflammatory bowel disease, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 16788–16793.
[166] L. Antonioli, M. Fornai, R. Colucci, N. Ghisu, F. Da Settimo, G. Natale, O. Kastsiuchenka, E. Duranti, A. Virdis, C. Vassalle, C. La Motta, L. Mugnaini, M.C. Breschi, C. Blandizzi, M. Del Taca, Inhibition of adenosine deaminase attenuates inflammation in experimental colitis, J. Pharmacol. Exp. Ther. 322 (2007) 435–442.
[167] V.L. Kolachala, T.S. Obertone, L. Wang, D. Merlin, S.V. Sitaraman, Adenosine 2b receptor (A2bR) signals through adenylate cyclase (AC) 6 isoform in the intestinal epithelial cells, Biochim. Biophys. Acta 1760 (2006) 1102–1108.
[168] B.P. Chandrasekharan, V.L. Kolachala, G. Dalmasso, D. Merlin, K. Ravid, S.V. Sitaraman, S. Srinivasan, Adenosine 2B receptors (A(2B)AR) on enteric neurons regulate murine distal colonic motility, FASEB J. 23 (2009) 2727–2734.
[169] V. Kolachala, B. Ruble, M. Vijay-Kumar, L. Wang, S. Mwangi, H. Figler, R. Figler, S. Srinivasan, A. Gewirtz, J. Linden, D. Merlin, S. Sitaraman, Blockade of adenosine A2B receptors ameliorates murine colitis, Br. J. Pharmacol. 155 (2008) 127–137.
[170] V.L. Kolachala, M. Vijay-Kumar, G. Dalmasso, D. Yang, J. Linden, L. Wang, A. Gewirtz, K. Ravid, D. Merlin, S.V. Sitaraman, A2B adenosine receptor gene deletion attenuates murine colitis, Gastroenterology 135 (3) (2008) 861–870.