Cutting Edge

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Cutting Edge: A2B Adenosine Receptor Signaling Provides Potent Protection during Intestinal Ischemia/Reperfusion Injury[1]

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Gastrointestinal ischemia/reperfusion (IR) injury significantly contributes to the morbidity and mortality of critical illness. In this study, we hypothesized a protective role for extracellular adenosine signaling in intestinal IR injury. Initial profiling studies of mucosal scrapings following murine IR demonstrated selective induction of the A2B adenosine receptor (A2BAR) transcript. Moreover, gene-targeted mice for the A2BAR showed more profound intestinal IR injury compared with controls. In contrast, A2AAR$^{-/-}$ mice exhibited no differences in intestinal injury compared with littermate controls. In addition, selective inhibition of the A2BAR resulted in enhanced intestinal inflammation and injury during IR. Furthermore, A2BAR agonist treatment (BAY 60-6583) protected from intestinal injury, inflammation, and permeability dysfunction in wild-type mice, whereas the therapeutic effects of BAY 60-6583 were abolished following targeted A2BAR gene deletion. Taken together, these studies demonstrate the A2BAR as a novel therapeutic target for protection during gastrointestinal IR injury. ***The Journal of Immunology,*** **2009, 182: 3965–3968.**

Transient abdominal ischemia caused by vascular disease, surgery, or organ transplantation leads to profound functional and structural alterations of the gastrointestinal tract (1). Intestinal ischemia/reperfusion (IR)[3] can proceed to a systemic response and may result in bacterial translocation, endotoxemia, acute respiratory distress syndrome, or acute hepatic injury (2). It has recently been appreciated that adenosine, a naturally occurring anti-inflammatory agent, represents an endogenous distress signal that modulates tissue damage and repair, particularly during conditions of hypoxia or ischemia (3). Four subtypes of adenosine receptors (ARs) have been characterized: A1AR, A2AAR, A2BAR, or A3AR (4). The A2AAR and A2BAR are coupled to stimulatory G proteins, resulting in increased cAMP concentrations, and have been associated with tissue protection and attenuation of inflammation (5, 6). In the present studies we sought to identify their contribution to cytoprotection during intestinal IR injury.

Materials and Methods

Gastrointestinal IR injury

Mice deficient in A2AAR (A2AAR$^{-/-}$; CD1 genetic background) (7) or A2BAR (A2BAR$^{-/-}$; C57BL/6 genetic background) (4) were compared with A2AAR$^{+/+}$ or A2BAR$^{+/+}$ littermates (wild type (WT)). Intestinal ischemia was produced by clamping the superior mesenteric artery for 15 min, followed by unclamping for 3 h of reperfusion (1). All injury parameters were measured following this time frame. In some experiments, mice were treated with saline (vehicle), PSB1115 (at 10 mg/kg administered i.v.; Tocris) or BAY 60-6583 (at 0.2, 1, or 2 mg/kg administered i.v.; Bayer) 10 min before IR.

Myeloperoxidase (MPO) activity

MPO activity was measured in intestinal and lung homogenates as described (1).

Serum enzymatic and cytokine measurements

Lactate dehydrogenase (Randox Laboratories), aspartate (AST) and alanine (ALT) aminotransferases (Teco Diagnostics), and IL-1 and IL-6 (R&D Systems) activities were measured as previously described (1).

Real-time RT-PCR

AR transcript levels were quantified by real-time RT-PCR using mucosal scrapings (1).

Western blotting

Proteins from mucosal scrapings were studied as described previously (1).

Intestinal permeability

Intestinal permeability was assessed by enteral administration of FITC-dextran 4000 (2 mg per 10 g of body weight; Sigma-Aldrich) 5 min before ischemia.

Histology

Intestinal histology was assessed as described previously (1).

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[3] Abbreviations used in this paper: IR, ischemia/reperfusion; AR, adenosine receptor; AST, aspartate aminotransferase; ALT, alanine aminotransferase; MPO, myeloperoxidase; WT, wild type.

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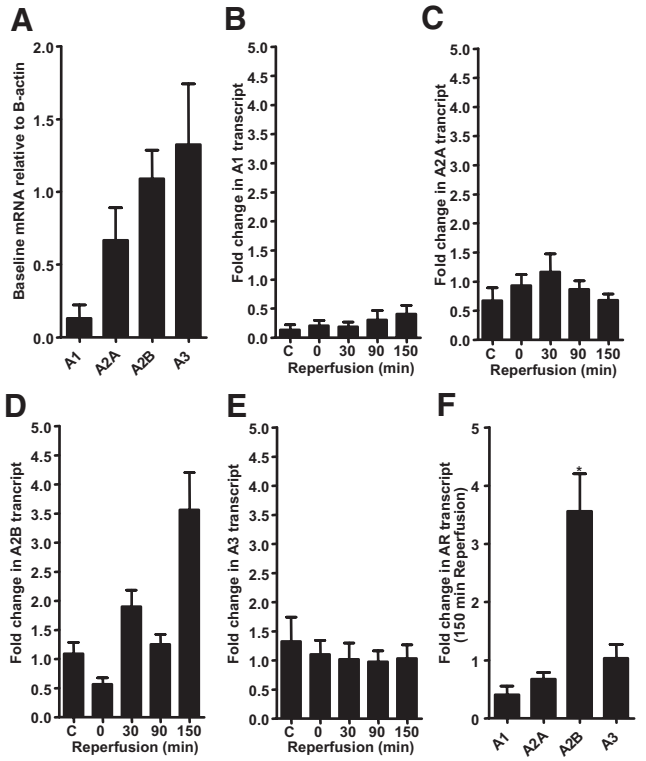

FIGURE 1. A2BAR mRNA is selectively induced by intestinal IR. *A*, Baseline mRNA following isolation of RNA from intestinal epithelial scrapings from WT mice calculated relative to β-actin and expressed as the mean ± SEM of three mice per group. *B–E*, A1 (*B*), A2A (*C*), A2B (*D*), and A3 (*E*) mRNA following 15 min of ischemia and 0–150 min of reperfusion expressed as fold change vs sham control (C) ± SEM of three mice per group. *, $p < 0.05$ vs sham control (C). *F*, AR mRNA following 15 min ischemia and 150 min reperfusion. *, $p < 0.05$ vs other groups.

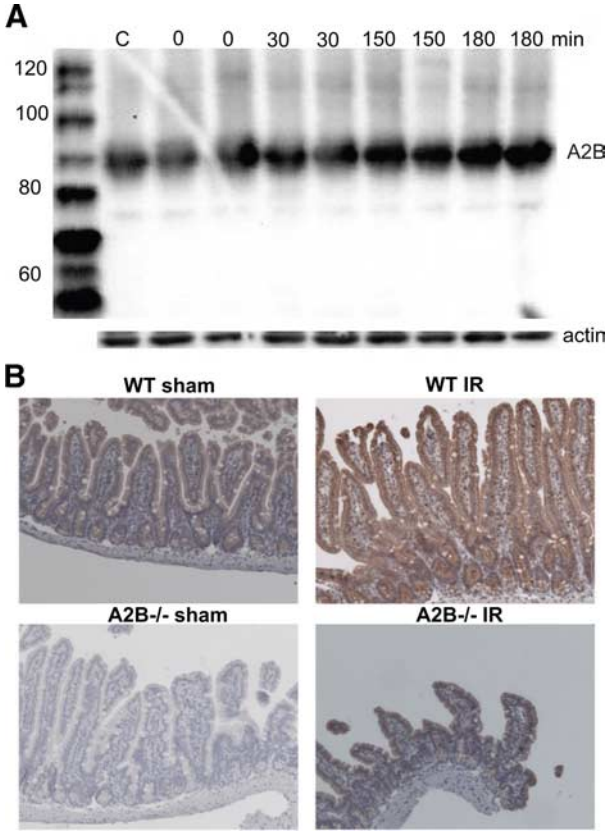

FIGURE 2. *A*, Western blot from intestinal epithelial scrapings from WT mice subjected to 15 min of ischemia and 0–180 min of reperfusion vs sham controls (C). Membranes were probed with anti-A2BAR Ab and reprobed for β-actin. *B*, Immunohistochemistry for A2BAR (original magnification ×100) (brown staining represents A2BAR-positive tissue). Control studies in $A2BAR^{-/-}$ mice ($A2B^{-/-}$). One representative slide of three independent experiments is displayed.

Statistical analysis

Injury score is presented as median with or without range and analyzed with a Kruskal-Wallis test. All other data are presented as mean ± SEM and analyzed using ANOVA.

Results

Intestinal IR is associated with selective induction of the A2BAR

As the first step, we investigated the transcriptional consequences of intestinal IR on AR expression. Baseline AR mRNA levels from mucosal scrapings are displayed in Fig. 1*A*. Transcriptional responses of ARs following 15 min of intestinal ischemia and different reperfusion times (0–150 min) are shown in Fig. 1, *B–E*. These studies revealed a selective induction of the A2BAR. Comparative transcript levels of all four ARs following intestinal IR injury demonstrate that the A2BAR becomes the dominant intestinal AR following injury (Fig. 1*F*). A2BAR induction during IR injury was confirmed on the protein level by Western blotting (Fig. 2*A*) and immunohistochemistry (15 min of ischemia, 3 h of reperfusion; Fig. 2*B*). The secondary Ab alone stained negative, and similar staining experiments in $A2BAR^{-/-}$ mice confirmed specificity for the A2BAR (data not shown). In contrast, no changes in A2AAR transcript levels were observed (data not shown). These studies suggest selective induction of the A2BAR transcript and protein following IR. This may be part of an endogenous anti-inflammatory pathway elicited by IR, as will be addressed in the following studies.

Intestinal IR injury is increased in $A2BAR^{-/-}$ mice

Both, A2AAR and A2BAR have been associated with tissue protection during limited oxygen availability (3, 5). In this study, we first investigated the role of A2AAR signaling in intestinal IR using $A2AAR^{-/-}$ mice. Consistent with previous studies (1), IR was associated with a significant increase in serum ALT (Fig. 3*A*), AST (Fig. 3*B*), and IL-6 (Fig. 3*C*) in WT ($A2AAR^{+/+}$) mice. $A2AAR^{-/-}$ showed similar injury as that of $A2AAR^{+/+}$ mice (Fig. 3, *A–C*). In contrast, studies in $A2BAR^{-/-}$ mice revealed that intestinal IR significantly increased serum ALT (Fig. 3*D*), AST (Fig. 3*E*), and IL-6 concentrations (Fig. 3*F*) in $A2BAR^{-/-}$ vs $A2BAR^{+/+}$ mice. These data provide genetic evidence for A2BAR-dependent gut protection against intestinal IR.

A2BAR inhibition accentuated injury following intestinal IR

To confirm the functional role of the A2BAR, we performed pharmacological studies in WT mice subjected to intestinal IR following treatment with the highly specific A2BAR antagonist PSB1115 (8) (at 10 mg/kg administered i.v.) or vehicle (saline). Following intestinal IR, PSB1115-treated mice demonstrated significantly higher ALT (Fig. 4*A*) and AST (Fig. 4*B*) levels and intestinal neutrophil infiltration (MPO) (Fig. 4*C*) vs vehicle-treated mice. PSB1115 treatment also enhanced intestinal injury in $A2AAR^{-/-}$ (supplemental Fig. 1).[4] These studies confirm our genetic studies and provide pharmacological evidence.

[4] The online version of this article contains supplemental material.

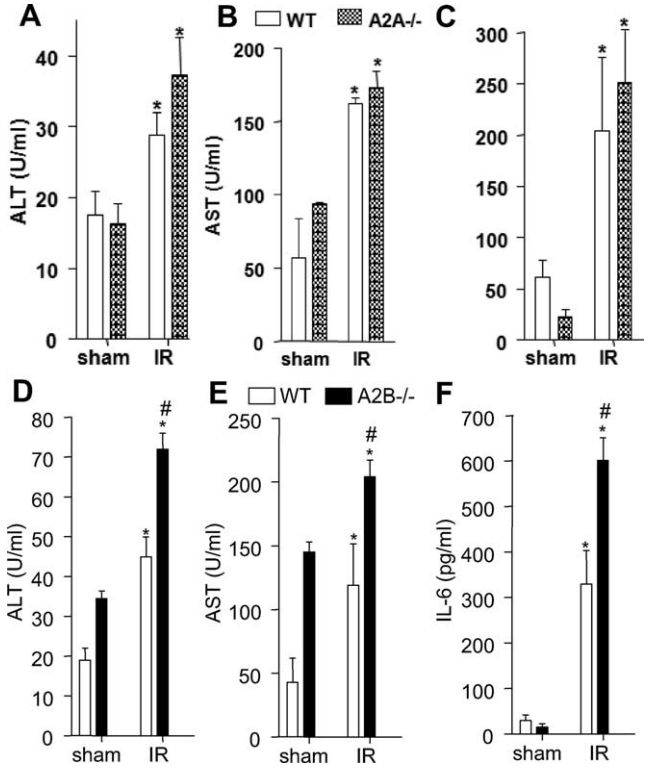

FIGURE 3. Injury is increased in A2BAR$^{-/-}$ but not A2AAR$^{-/-}$ mice. A2AAR-deficient (A2A$^{-/-}$) mice (*A–C*) or A2BAR-deficient (A2B$^{-/-}$) mice (*D–F*) vs littermate controls (WT) were subjected to 15 min of ischemia and 3 h of reperfusion. ALT (*A* and *D*), AST (*B* and *E*), and IL-6 (*C* and *F*) were expressed as the mean ± SEM of 4–12 mice per group. *, $p < 0.05$ vs the respective sham group; #, $p < 0.05$ vs WT IR.

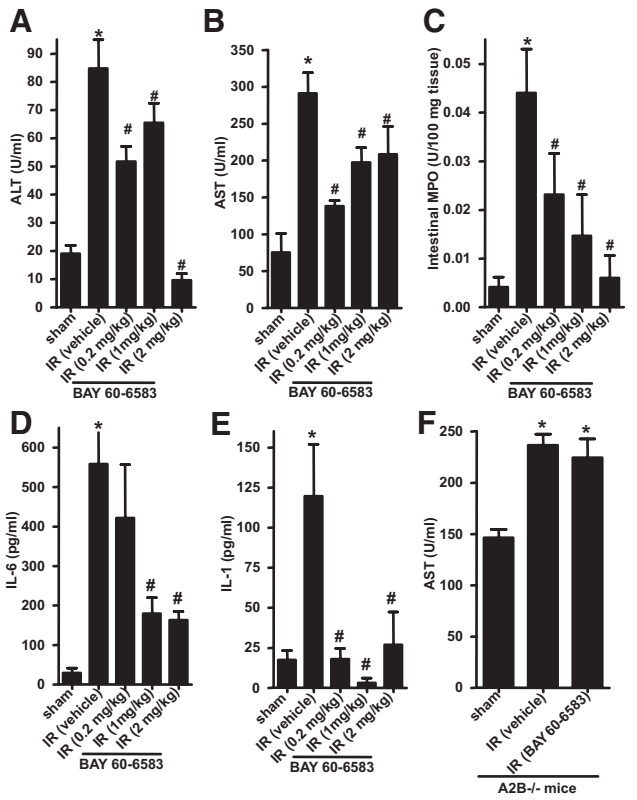

FIGURE 5. Treatment with an A2BAR agonist, BAY 60-6583, decreases injury in WT but not A2B$^{-/-}$ mice. WT mice were treated with saline (vehicle), 0.2, 1, or 2 mg/kg BAY 60-6583 (administered i.v. 10 min before induction of ischemia) and subjected to 15 min of ischemia and 3 h of reperfusion. *A–E*, ALT (*A*), AST (*B*), intestinal MPO (*C*), IL-6 (*D*), and IL-1 (*E*). A2B$^{-/-}$ mice were treated with vehicle or 2 mg/kg BAY 60-6583 and subjected to IR. *F*, Serum AST. Results are expressed as mean ± SEM of 4–12 mice per group. *, $p < 0.05$ vs sham control; #, $p < 0.05$ vs IR (vehicle).

Treatment of WT but not A2BAR$^{-/-}$ mice with a selective A2BAR agonist attenuated intestinal IR injury

Next, we pursued a potential therapeutic role of A2BAR signaling, using a previously described selective A2BAR agonist (BAY 60-6583) (4, 9, 10). WT mice were treated with 0.2, 1, or 2 mg/kg (i.v.) BAY 60-6583. As shown in Fig. 5, *A–E*, treatment with the selective A2BAR agonist resulted in a significant attenuation of injury. To demonstrate specificity of the A2B agonist, we treated A2B$^{-/-}$ mice with BAY 60-6583. In contrast to WT mice, BAY 60-6583 treatment (at 2 mg/kg administered i.v.) before intestinal IR failed to attenuate injury in A2BAR$^{-/-}$ mice (Fig. 5*F*). Moreover, BAY 60-6583 also provided intestinal protection in A2AAR$^{-/-}$ mice (supplemental Fig. 2), and the protective effects persisted if BAY 60-6583-treatment was initiated immediately following ischemia (supplemental Fig. 3). Because mucosal barrier dysfunction is commonly associated with intestinal IR (2) we next examined mucosal barrier dysfunction during intestinal IR injury. WT mice were treated with BAY 60-6583 before IR. We found a significant reduction in intestinal permeability vs vehicle-treated mice (Fig. 6*A*). Furthermore, histological analysis revealed that the observed IR-induced injury in vehicle-treated mice was reduced with A2BAR agonist pretreatment (Fig. 6, *B* and *C*).

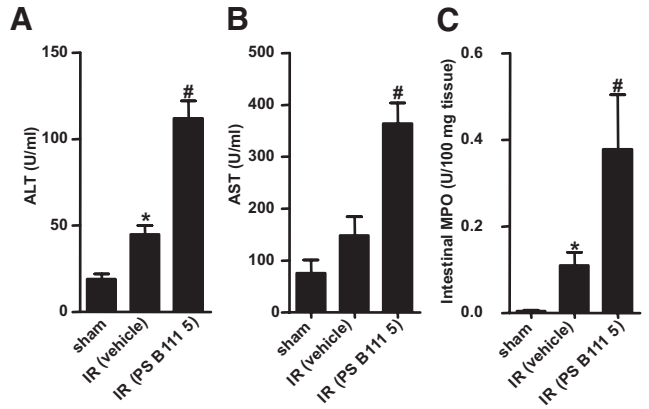

FIGURE 4. Inhibition of the A2BAR by PSB1115 increases injury. WT mice were treated with PSB1115 (at 10 mg/kg administered i.v. 10 min before induction of ischemia) or saline (vehicle) and subjected to 15 min of ischemia and 3 h of reperfusion. ALT (*A*), AST (*B*), and intestinal MPO (*C*) were expressed as the mean ± SEM of 8–12 mice per group. *, $p < 0.05$ vs sham; #, $p < 0.05$ vs IR (vehicle).

Discussion

Together, our findings indicate A2BAR as a potential therapeutic target during intestinal IR injury. As such, transcriptional profiling of intestinal tissue revealed a selective induction of the mucosal A2BAR with IR. In addition, we found that gene-targeted deletion or selective inhibition of the A2BAR results in significantly higher levels of intestinal IR injury. Furthermore, A2B agonist treatment provided strong protection from intestinal inflammation and permeability dysfunction during IR injury.

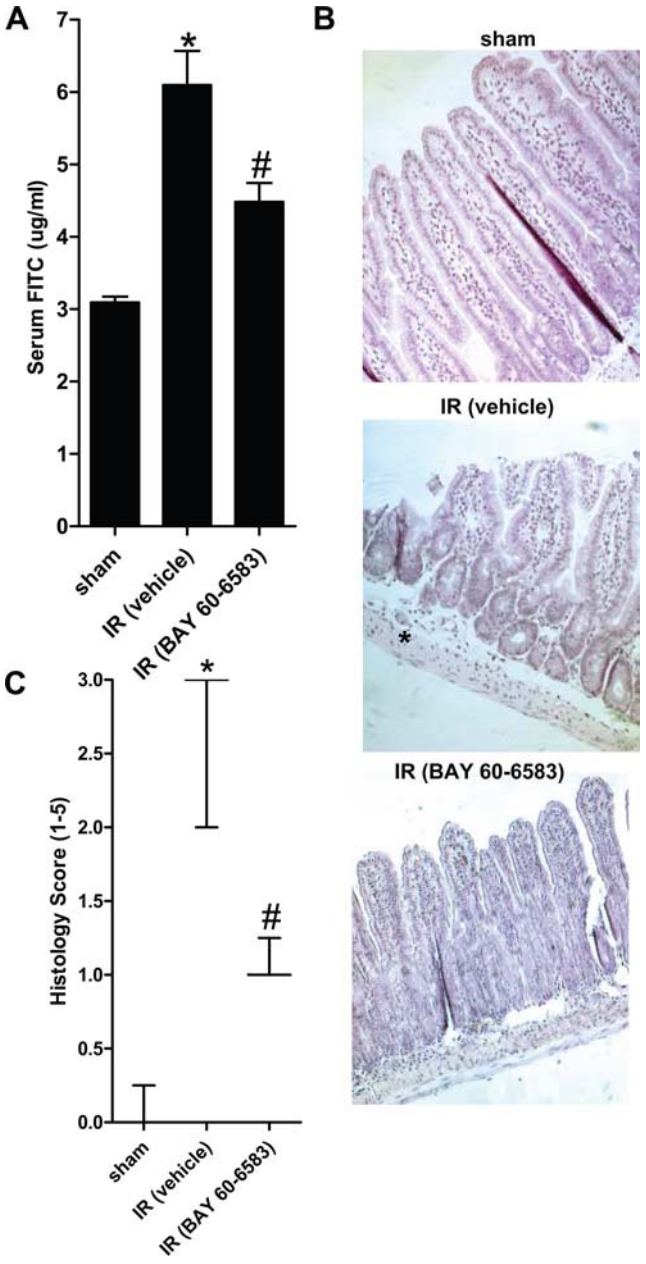

FIGURE 6. Treatment with the A2BAR agonist decreases intestinal permeability and histological injury. WT mice were treated with saline (vehicle) or 2 mg/kg BAY 60-6583 (administered i.v. 10 min before induction of ischemia) and subjected to IR (15 min of ischemia and 3 h of reperfusion). *A*, Intestinal permeability to FITC 4000 (following enteral administration of 2 mg per 10 g of body weight 5 min before ischemia) expressed as the mean ± SEM of five mice per group. *, $p < 0.05$ vs sham. #, $p < 0.05$ vs IR (vehicle). *B*, H&E staining of jejunum sections (original magnification ×200). *C*, Quantification of ischemic injury ($n = 6$ mice per group expressed as median ± range).

The present studies are consistent with previous work showing a protective effect of A2BAR signaling in models of acute inflammation (11). For example, a recent study demonstrated that attenuation of mucosal inflammation during hypoxia involves A2BAR signaling events (11). Similarly, studies of acute vascular injury using a femoral artery injury model revealed that the A2BAR prevents vascular lesion formation in an injury model that resembles human restenosis after angioplasty (12). Other studies have found a role for A2AAR in intestinal protection during inflammation (13). A possible explanation of A2BAR-dependent protection could be related to high expressional levels of the A2BAR following IR exposure. In fact, previous studies had shown high transcriptional responsiveness of the A2BAR to different stimuli (4, 8–10, 14).

In summary, the present studies suggest A2BAR signaling in tissue protection during intestinal IR injury. Further challenges will include the translation from "mice to man". Moreover, potential side effects of A2BAR agonist treatment, for example on platelet function or bleeding time (15), will have to be investigated.

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Disclosures

The authors have no financial conflict of interest.

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